7-2021

Survival Kinetics and Antimicrobial Interventions to Mitigate Foodborne Pathogen Contamination of Low-Moisture Foods

Rajat Sharma
The University of Texas Rio Grande Valley

SURVIVAL KINETICS AND ANTIMICROBIAL INTERVENTIONS TO MITIGATE FOODBORNE PATHOGEN CONTAMINATION OF LOW-MOISTURE FOODS

A Thesis

by

RAJAT SHARMA

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

July 2021

Major Subject: Biochemistry and Molecular Biology

SURVIVAL KINETICS AND ANTIMICROBIAL INTERVENTIONS TO MITIGATE FOODBORNE PATHOGEN CONTAMINATION OF LOW-MOISTURE FOODS

A Thesis
by
RAJAT SHARMA

COMMITTEE MEMBERS

Dr. Veerachandra K. Yemmireddy
Chair of Committee

Dr. Debasish Bandyopadhyay
Committee Member

Dr. Daniele Provenzano
Committee Member

July 2021

ABSTRACT

Sharma, Rajat, Survival Kinetics and Antimicrobial Interventions to Mitigate Foodborne Pathogen Contamination of Low Moisture Foods. Master of Science (MS), July, 2021, 65 pp., 6 tables, 8 figures, 129 references.

Contamination of low-moisture foods (LMFs) with foodborne pathogens such as *Salmonella* and Shiga-toxin producing *Escherichia coli* (STEC) has been rising over the last couple of years. Commodity groups such as peanuts, tree nuts, and their products are frequently recalled when associated with illness outbreaks resulting from these pathogens. In this project, two major challenges related to understanding the survival kinetics of pathogens and the efficacy of novel antimicrobial treatments in mitigating their risk in LMFs were systematically investigated. Studies were conducted for determining the survival kinetics of three *Salmonella* spp. strains in three different tree nut flours (i.e., almond, chestnut, and hazelnut flours). The effect of storage relative humidity (RH): 25, 45, and 70% on the survival kinetics was determined over a period of 120 days at 21±2ºC. Results indicate that the type of tree nut flour and storage RH has a significant ($P \leq 0.05$) effect on survival. *Salmonella* survived better at lower RH (25 or 45%) compared to higher RH (70%) conditions. The effect of flour composition on the survival of *Salmonella* needs to be further investigated. In another study, the efficacy of UV-C treatment in comparison to heat treatment to mitigate the risk of *Salmonella* and STEC was determined using peanut flour as a model food. Hot-water treatment resulted in a 3 to 4 log

CFU/g reduction of tested pathogens. Raising the treatment temperature from 60 to 80°C and increasing the treatment time from 1 to 30 min increased the log reductions. UV-C treatment was found to be equally (if not more) efficacious in reducing pathogen CFUs. A reduction from 2 to 3 log CFU/g was observed after a 60 min treatment time. Weibull model best predicted the thermal death and UV inactivation kinetics of tested pathogens in peanut flour. Further studies should be focused on increasing the UV-C efficacy on par with heat treatment and determining the effect of these treatments on quality characteristics of tested LMFs.

Keywords: Low-moisture foods; Foodborne pathogens; UVC; Predictive models; Survival kinetics; Inactivation.

DEDICATION

I dedicate this thesis to my mother Mrs. Savita Sharma, my father, Mr. Surinder Sharma, my sisters, Himani Sharma and Harsimran Kapoor, my girlfriend Dimple Sharma, my uncle Mr. Surinder Arora, my brotherly friends Amandeep Singh, and Gurjot Singh. I am highly thankful for your love, support and motivation which encouraged me to complete my master's thesis.

ACKNOWLEDGMENT

Firstly, I am thankful to my thesis advisor and mentor, Dr. Veerachandra K. Yemmireddy for his persistent support and guidance through the time of research. He has been very helpful while designing and conducting the experiments. I will always remember his contribution towards my career. I am glad that I joined his lab. I am greatful to my committee members Dr. Daniele Provenzano and Dr. Debasish Bandyopadhyay for serving on my committee and their needed support towards my thesis especially during committee meetings. I also give special thanks to Dr. Robert Dearth for being an effective program coordinator and helping the course transfer process from my previous school and for providing invaluable advice on any matters related to academics. I am highly indebted to the Graduate College at UTRGV for awarding me Presidential Graduate Research Assistantship to pursue my graduate studies. I would like to thank Dr. Priti Sharma for her immense support through my time as a graduate student. My family and friends need to be mentioned for unwavering support during this journey and my friends Amandeep Singh and Asfakur Rahman for making lab environment friendly enough to work enthusiastically. Lastly, I am grateful to my childhood friends Shubham Arora and Rahul Saran.

TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGMENT vi

TABLE OF CONTENTS vii

LIST OF TABLES… ix

LIST OF FIGURES x

CHAPTER I. INTRODUCTION AND LITERATURE REVIEW 1

Abstract 1

Introduction 2

Low-moisture foods (LMFs) 2

Pathogens of concern in LMFs 3

Salmonella spp. 4

Escherichia coli O157:H7… 5

Causes of foodborne pathogen contamination in LMFs… 5

Challenges in inoculation and recovery 6

Survival kinetics during storage 7

Development, verification and validation of antimicrobial interventions 8

Aims 10

Overall hypothesis 10

Objectives 10

Summary 11

CHAPTER II. EFFECT OF STORAGE RELATIVE HUMIDITY ON THE SURVIVAL KINETICS OF *SALMONELLA* SPP., IN DIFFERENT TREE NUT FLOURS 16

Abstract 16

Introduction 17

Materials and Methods 18

Results 21

Discussion 24

CHAPTER III. THERMAL AND UV-C INACTIVATION KINETICS OF *SALMONELLA* AND *E. COLI* O157:H7 IN PEANUT FLOUR 29

Abstract 29

Introduction 29

Materials and Methods 32

Results and discussion 37

Conclusions 42

CHAPTER IV. CONCLUSIONS AND FUTURE DIRECTIONS. 50

REFERENCES 54

BIOGRAPHICAL SKETCH 65

LIST OF TABLES

Page

Table 1.1: Selected list of foodborne pathogen outbreaks and recalls on low-moisture foods……. 12

Table 1.2: Studies on survival kinetics of foodborne pathogens on low-moisture foods under storage…….13

Table 1.3: Selected list of inactivation studies on low-moisture foods……14

Table 1.4: Effect of inactivation methods on properties of LMFs……15

Table 2.1: Comparison of survival kinetics of *Salmonella* spp., across different variables……....28

Table 3.1: Parameter estimates of log-linear and Weibull models in predicting the inactivation kinetics…….....49

LIST OF FIGURES

Page

Figure 2.1: Changes in A_w of different tree nut flours when stored at different RH conditions……26

Figure 2.2: Survival of *Salmonella* spp., on (A) almond, (B) chestnut, and (C) hazelnut flours at 25, 45, and 70% RH, respectively……27

Figure 3.1: Thermal treatment experimental set-up……...43

Figure 3.2: UV-C treatment experimental set-up using custom-built UV-C chamber……..44

Figure 3.3: Thermal inactivation of *Salmonella* spp., in peanut flour at 60, 70, and 80°C and vertical bars represent standard deviation……...45

Figure 3.4: Thermal inactivation of *E. coli* O157:H7 in peanut flour at 60, 70, and 80°C and vertical bars represent standard deviation……46

Figure 3.5: UV-C inactivation of *Salmonella* spp., and *E. coli* O157:H7 in peanut flour and vertical bars represent standard deviation……47

Figure 3.6: Effect of temperature on 1 log CFU/g reduction times of *Salmonella* spp., and *E. coli* O157:H7 in peanut flour is represented using linear regression lines…….....48

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Abstract

Background

Low-moisture foods (LMFs), which were generally considered microbiologically safe, now have escalated foodborne pathogens outbreak risk of foodborne pathogens.

Objective

The main objectives of this chapter are: (1) To understand the risk of microbial contamination in LMFs; (2) To identify the major pathogens of concern, (3) To identify factors promoting the contamination, (4) To summarize current scientific understanding and identify knowledge gaps, (5) To develop and devise research hypothesis and objectives

Results

Salmonella spp. was found to be the major pathogen of concern in LMFs followed by Shiga toxin-producing *E. coli*. Several factors those including water activity, storage conditions and food type were found to affect the survival of pathogens on or in LMFs.

Conclusion

The lack of sufficient data to understand the survival characteristics of *Salmonella* in low water activity/moisture foods like tree nuts and their products, as well as efficient strategies to mitigate their risk in those products, warrants further research.

Introduction

Microbial contamination with foodborne pathogens has now become a recurring issue of food safety. The U.S. Centers for Disease Control and Prevention (CDC) data predicted that close to 50 million people (i.e. 1 in 6 Americans) fall sick, and 3000 die every year in the United States due to foodborne diseases, accounting for more than $ 77 billion losses annually (T. J. A. m. Bintsis, 2017; Scharff, 2012). Over the years, more than 250 disease-causing pathogens have been found to contaminate variety of foods causing several foodborne pathogen outbreaks. These include but are not limited to *Campylobacter jejuni*, *Salmonella* spp., *Shigella* spp., *Clostridium botulinum*, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, Noroviruses, and Hepatitis A virus. Most notably, *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), and *Listeria monocytogenes* are notorious for most of numerous high-profile foodborne disease outbreaks. Microbial contamination by foodborne pathogens occurs at various stages in the food chain ranging from production to distribution (Frelka & Harris, 2014). A variety of foods function as vehicles by these pathogens to cause foodborne illnesses. Among these, foodborne outbreaks through low moisture food (LMF) products have seen a dramatic increase recently and pose a significant challenge to protect public health.

Low-moisture foods

Low-moisture foods (LMFs), also known as low water activity foods (LWAFs), are comestibles whose water activity (A_w) is lower than or equal to 0.7 (T. Blessington, C. G, Theofel, E. J. Mitcham, & L. J, Harris, 2013b; Enache, Podolak, Kataoka, & Harris, 2017). Water in food is present in two forms: (i) Free or active water available for the chemical, enzymatic and biological reactions, and (ii) Bound water which is unavailable as a solvent, and removal of it causes significant damage to food tissues. Water activity (A_w) is a actually the free water content

in foods and is represnted as the vapor pressure of food divided by the vapor pressure of distilled water under identical conditions (FDA, 2014). This means A_w of any food is less than 1 and pure water will have an A_w of 1. Moisture content is a representation of free and bound water contents. Thus, even humid foods may harbor low A_w as the moisture can be bound by solutes such as sucrose and sodium chloride preventing evaporation (Gurtler, Doyle, & Kornacki, 2014). Some LWAFs have a low A_w naturally, while others become LWAF upon processing and removal of water. For convenience, low water activity/moisture foods will be collectively referred to LMFs in this thesis which is the popular term that is used in literature (Beuchat et al., 2013b; Gurtler et al., 2014). Few common examples of LMFs include but are not limited to different types of tree nuts, peanuts, seeds, spices, and their processed products as well as dairy, confectionary, dried meat, fish, and related products (Cordier, 2014; Gurtler et al., 2014; Kornacki & Desautels, 2014; Pinkas & Keller, 2014).

Pathogens of concern in LMFs

Several pathogens have been found to persist in association with a variety of LMFs as vehicle and reach the human gut causing different foodborne diseases in several developed and developing countries, including the United States, Canada, Germany, India, Australia, Brazil, and New Zealand (Gurtler et al., 2014). These pathogens are mostly bacterial, although some LMF outbreaks have been associated with viral pathogens as well. Among prokaryotes *Salmonella* spp., STEC, *Yersinia* spp., *Campylobacter jejuni*, *Cronobacter*, *Staphylococcus aureus*, *Listeria monocytogenes, Clostridium botulinum*, *Enterobacter* spp., *Shigella* spp., *Aspergillus* spp., feature prominently (Beuchat et al., 2013b). Among these, *Salmonella* spp., have been found to pose the highest risk to LMFs followed closely by pathogenic *E. coli* (Beuchat et al., 2013b; Farakos & Frank, 2014; Gurtler et al., 2014). A wide range of LMFs,

including spices, dried dairy-based products, edible seeds and nuts, powdered infant formula, different types of flours, are implicated in a number of outbreaks (Gurtler et al., 2014). A comprehensive list of these outbreaks and associated pathogens were reported in Table 1.1. The general characteristics and pathogenicity of the two most problematic pathogens in LMFs was detailed below.

***Salmonella* spp.**

Salmonella is a facultative gram-negative anaerobic rod falling under the family *Enterobacteriaceae* with approximately 2-5µm in size (D'Aoust & Maurer, 2007; Popoff, Le Minor, & Bacteria, 2015). *Salmonellae* is a mesophilic organism, an optimum growth temperature in the range of 30 to 39°C but can tolerate low (20°C) and high (45°C) temperatures as well. These bacteria remain viable in pH conditions ranging from 3.9 – 9.5 with an optimum growth at pH 6.5 – 7.5 (Podolak, Enache, Stone, Black, & Elliott, 2010; Strawn, 2009). Serological identification of *Salmonella* is carried out by agglutination of *Salmonella* antigens: flagellin and lipopolysaccharides antigens, capsular antigens (D'Aoust & Maurer, 2007; Strawn, 2009). Virulence proteins are coded by gene clusters known as *Salmonella* pathogenicity islands (SPIs) and are classified as SPI-1and SPI-2 (Lahiri et al., 2010; Ly & Casanova, 2007). SPI genes are responsible for *Salmonella* entry and intracellular survival. Upon ingestion, *Salmonellae* promote their own phagocytosis using effector proteins released into host cell cytoplasm that modify actin cytoskeleton and remain in *Salmonella* containing vacuole (SCV) where they multiply (Ly & Casanova, 2007). Besides the type of microorganism, the infectious dose of a foodborne pathogen infectious dose depends on the immune system of the host as well (Unlu, 2021). For example, 10 - 50 cells in chocolate and 0.45 CFU/g in paprika have resulted in *Salmonella* illness outbreaks in those respective products (Greenwood & Hooper, 1983;

Lehmacher, Bockemühl, Aleksic, & Infection, 1995).

Escherichia coli O157:H7

Escherichia coli belongs to a class of bacteria that are usually non-pathogenic in nature and are commonly found among the microflora of animal and human intestinal tracts in a symbiotic relationship (Gyles, 2007; Kaper, Nataro, & Mobley, 2004; Nataro & Kaper, 1998). Common *E. coli* strains rarely cause infections except in case of individuals with weak immune systems. Nonetheless, several strains have attained specific virulence attributes which enable them to cause a wide range of diseases (Meng et al, 2007). The isolates are differentiated on the basis of three major antigens which enable serotyping: O (somatic), H (flagella), and K (capsule) antigens (Meng et al, 2007). *E. coli* O157:H7 is one such serotype that gets its name due to the production of 157^{th} somatic (O) antigen and 7^{th} flagellar (H) antigen (Mead & Griffin, 1998). *E. coli* O157:H7 expresses Shiga-like toxin that resembles the toxin produced by *Shigella* spp., and therefore called as Shiga toxin-producing *E. coli* (STEC) (Gyles, 2007; Paton & Paton, 1998). Ingested STEC survives in the low pH environment of the stomach and is presumed to reach terminal ileum and colon and where it attaches to enterocytes and colonizes the intestinal mucosa causing characteristic lesions. Shiga toxin produced in the colon causes damage to microvilli and blood vessels, resulting in bloody diarrhea during severe infections and results in hemolytic uremic syndrome (HUS) also known as kidney failure (Tarr, Gordon, & Chandler, 2005). In 2017, an outbreak of *E. coli* O157:H7 on Soy nut butter infected 32 people from 12 states, and 12 infected persons developed HUS (CDC, 2017). Fewer than 100 STEC cells are enough to induce an illness (Matthews et al, 2017).

Causes of foodborne pathogen contamination in LMFs

Although current understanding on foodborne pathogen contamination in LMFs is very

limited, several factors such as contaminated ingredients, cross-contamination in the processing environment, poor equipment design and improper sanitation practices, and lack of proper environmental monitoring programs contributed to known outbreaks and recalls in the past (Beuchat et al., 2013b; Gurtler et al., 2014). For example, *Salmonella* was found in herbal tea and snack preparations when contaminated aniseed and seasoning mix are used as ingredients in these products (CDC, 2007a; Koch et al., 2005). Similarly, the outbreak of *Salmonella* on halvah candy was attributed to cross-contamination during processing (Brockmann, Piechotowski, & Kimmig, 2004; Podolak et al., 2010). Contamination of infant dried milk, peanut butter, and chocolate with *Salmonella* occurred due to damaged spray dryer, roof leakage, and leaking pipes, respectively (Carroll, 2016; Funk, 2007; Rowe et al., 1987). These types of issues are not just limited to LMFs but are very common in other food categories as well. However, based on the literature review conducted as part of this project, the major scientific challenges to understand and address the issue of foodborne pathogens in LMFs boils down to three major categories: (1) Methods to inoculate, recover and detect pathogens in LMFs, which simulate real-life contamination scenarios, (2) Understanding the survival kinetics of pathogens during storage and the effect of different environmental conditions, (3) Development, verification and validation of novel intervention strategies to mitigate the risk. These areas were briefly elaborated below:

Challenges in inoculation and recovery

Two approaches of inoculation: (i) Wet and (ii) Dry inoculation methods are commonly followed in LMFs to simulate contamination under wet or dry conditions (Xu et al., 2020). Wet inoculation methods involve the use of aqueous suspension of pathogen, while dry inoculation uses a carrier agent such as glass beads, talc, sand, and chalk powder for transferring the inoculum to the LMFs. Carriers are first seed inoculated with wet inoculum and then added to

bulk food material to simulate general contamination events from dry processing environments (Blessington et al., 2013b; Xu et al., 2020). Though these two methods were used quite extensively used in various scientific studies, these methods have their own advantages and disadvantages. For example, preparation time for wet inoculum is lower in comparison to dry inoculum (Blessington et al., 2013b). Wet inoculation is relatively simple to achieve the same level of pathogen concentration, but it may not reflect true contamination scenarios observed in real life. Furthermore, wet inoculation induces the formation of clumps and promotes LMFs stickiness, prevents even distribution of pathogen, and causes quality losses (Xu et al., 2020). While it is a bit difficult to achieve and estimate desired inoculum level in the dry inoculation approach (Enache et al., 2015; Hildebrandt et al., 2017) but it may be well suited to reflect the contamination from the dry environment, which is most likely observed in LMF processing areas. Recovery of the pathogen is another challenge in LMF studies. General microbiological recovery techniques such as serial dilutions in suitable broth medium to maintain the viability of pathogens followed by plating and incubation are commonly followed. However, LMFs such as spices possesses natural antimicrobial compounds which inactivate pathogens during sample processing and recovery (Ceylan, Fung, & Microbiology, 2004; Cordier, 2014; Nychas, 1995). Therefore, higher dilutions and addition of neutralizing agents such as K_2SO_3 and additional enrichment times are recommended for improved pathogen recovery (Blessington et al., 2013b; Gurtler et al., 2019).

Survival kinetics during storage

LMFs, in general, is shown not to support the growth of foodborne pathogens. However, pathogens like *Salmonella enterica* have been found to convert into a viable but non-culturable state (VBNC) and survive for prolonged time in LMF conditions (Kusumoto, Asakura et al.

2012). A return to a vegetative state upon exposure to moist environmental conditions has been reported to increase their numbers (Blessington et al., 2013b; Hills, Manning, Ridge, & Brocklehurst, 1997; M. S. Nascimento et al., 2018). Table 1.2 shows various environmental factors influencing the survival kinetics of different foodborne pathogens. These factors include type of food matrix (Salazar et al., 2019b), A_w (Ballom, Tsai, Taylor, Tang, & Zhu, 2020), temperature (Forghani et al., 2019b), inoculum density (Beuchat, Mann, Kelly, & Ortega, 2017), sugar and nutrient availability (M. S. Nascimento et al., 2018), temperature and relative humidity (RH) conditions in storage environment (Ballom et al., 2020; Salazar et al., 2019b), type of pathogen (Blessington, Mitcham, & Harris, 2012), and length of storage period (Forghani et al., 2019b; M. S. Nascimento et al., 2018).

Development, verification, and validation of antimicrobial interventions

Several treatments methods for inactivating foodborne pathogens in the past have been developed and tested on wide range of LMFs. These inactivation strategies largely classified into thermal and non-thermal methods. Thermal methods of inactivation includes dry heat treatment, infrared heating, radiofrequency, microwave heating, and instant controlled pressure drop technology (Anderson, 2019; Rifna, Singh, Chakraborty, & Dwivedi, 2019). Non-thermal methods of inactivation includes high-pressure processing, non-thermal plasma, pulsed light, irradiation, ozone processing, and UV light treatment (Anderson, 2019; Rifna et al., 2019). As reported in Table 1.3, inactivation caused by thermal and non-treatment varies depending upon several factors such as time of treatment, intensity of treatment, type of food matrix, pathogen type, temperature of treatment, and water activity. Exposure of micro-organisms to heat causes denaturation of essential cellular components such as proteins, ribosomes, enzymes, RNA, and cell membrane. As a result, the cell is unable to replicate and sustain function, leading to lysis,

death or sublethal injury from which cells may not recover (Gomez, 1977; Nguyen, Corry, Miles, & Microbiology, 2006; Smelt, Brul, & nutrition, 2014). Heat treatment is widely used to curtail the risks posed by foodborne pathogens and several predictive models have been developed that help supply heat treatment doses required to inactivate specific pathogens (Smelt et al., 2014). Alternatively, UV treatment has shown promise as a non-thermal treatment to kill or inactivate pathogens. Traditionally, UV irradiation has been used for water treatment, surface decontamination, and air disinfection with limited food-related applications (Koutchma & Technology, 2009). However, the use of UV irradiation for applications in the food industry has seen increased interest in the last two decades. Studies have shown the potential of UV light for inactivating foodborne pathogens (T. Bintsis, Litopoulou-Tzanetaki, Robinson, & Agriculture, 2000; Gayán, García-Gonzalo, Álvarez, & Condón, 2014). UV light was proven to be effective against bacteria and viruses (Eischeid, Meyer, Linden, & Microbiology, 2009), parasites (Hijnen, Beerendonk, & Medema, 2006), fungi (Gomez-Lopez, Devlieghere, Bonduelle, & Debevere, 2005; Syamaladevi et al., 2015).

UV light belong to electromagnetic spectrum ranging from 200 to 400 nm. It is mainly subdivided into three regions by wavelength: UV-C (200 to 280 nm), UV-B (280 to 320 nm); and UV-A (320 to 400 nm). The maximum effectiveness of UV light is at a wavelength of about 254 nm (W. J. Kowalski, W. Bahnfleth, & M. T. J. I. n. Hernandez, 2009a; W. J. Kowalski, W. P. Bahnfleth, & M. T. J. I. N. Hernandez, 2009b). The absorption of UV-C light prompts the DNA photoproducts formation like pyrimidine 6-4 pyrimidine photoproducts and cyclo-butane pyrimidine dimers which obstruct transcription and replication leading to mutagenesis and cell death (Gayán et al., 2014). Disinfection efficacy of UV light is presented by the product of intensity (mW/cm^2) and the exposure time (s) and is commonly expressed as $mW\text{-}s/cm^2$ or

mJ/cm^2 and therefore depends on its fluency or dose delivered (Kowalski et al., 2009a). However, various physiochemical and sensory changes such as solubility, viscosity, water holding capacity, and palatability have been reported to occur in LMFs during UV light treatments (Table 1.4).

This project was conducted to achieve the following aims and objectives.

Aims

The aims of this project include: (i) determining the effect of RH on survival kinetics of *Salmonella* spp., (ii) verifying the efficacy of heat and UV-C treatments in *Salmonella* spp., and *E. coli* O157:H7 killing. As discussed earlier, relevant studies on different LMFs have been performed, but information on the effect of RH on *Salmonella* during storage of tree nut flours is scarce. In addition, the efficacy of UV-C treatment when tested against powered LMFs is not well studied. Survival and inactivation data will help to estimate the risk and developing quantitative microbial risk analysis (QMRA) models when subjected to novel treatment interventions.

Overall hypothesis

Survival of *Salmonella enterica* in tree nut flours at ambient temperature is negatively influenced by increasing storage RH while UV-C and hot water treatment decrease bacterial viability proportionate of dose and temperature increases.

Objectives

1. To determine the survival kinetics of *Salmonella* spp., on almond, chestnut, and hazelnut flour at different RH under extended storage time.
2. To determine the efficacy of hot water treatment and UV-C to inactivate *Salmonella* spp., and *E. coli* O157:H7 on peanut flour.

Summary

Increased frequency of foodborne pathogen outbreaks and recalls on LMFs prompted an investigation and identify major knowledge gaps to address the issues of survival kinetics and the development of inactivation methods. Survival of pathogens in LMFs and their mitigation was found to depend on multiple factors such as food A_w (Tsai et al., 2019), storage conditions (Salazar et al., 2019), microorganism (Koseki, Nakamura, & Shiina, 2015), type of treatment conditions (Jeong, Baik, & Kang, 2017), etc. However, the effect of these factors needs to be evaluated based on the specific food commodity in question. Therefore, studies are warranted to understand the influence of these variables on the survival kinetics of most problematic pathogens in LMFs and devise novel strategies to mitigate their risk and protect public health. This thesis work, organized into four chapters, is one step towards this goal and contributes towards filling knowledge gaps. The current introductory chapter (I) states the problem and highlights knowledge gaps, aims, hypotheses, and objectives constructed based on the reported literature. Chapter II evaluated the survival kinetics of *Salmonella* spp. in tree nut flours and the effect of storage RH. In chapter III, the efficacy of heat and UV-C were evaluated against the *Salmonella* spp. and *E. coli* O157:H7 on peanut flour. Finally, Chapter IV summarizes and draws conclusions from this research work.

Table 1.1 Selected list of foodborne pathogen outbreaks and recalls on low-moisture foods.

Type of pathogen	Food source	Number affected	Recall information	Year	Location	Reference
Salmonella Tennessee	Powdered infant formula	3		1993	United States, Canada	(Control & Prevention, 1993)
Salmonella Senftenberg	Baby cereal	8		1995	England	(Rushdy et al., 1998)
S Enteritidis PT 30	Almonds	168	70-90% of the product	2000 – 2001	Canada, United States	(Isaacs et al., 2005)
Salmonella Enterica	Peanut products	109		2001	Australia, Canada, England & Wales, and Scotland	(Kirk, Little, Lem, Fyfe, Genobile, Tan, Threlfall, Paccagnella, Lightfoot, Lyi, et al., 2004)
S Enteritidis PT 9c	Almonds	29	13 million pounds	2003 – 2004	Canada, United States	(Control & Prevention, 2004)
S Typhimurium PT 42	Raw flour	79		2008	New Zealand	(McCallum et al., 2013)
S Typhimurium	Peanut butter	714 (9 deaths)	3900 products	2009	United States	(Chang, Sreedharan, & Schneider, 2013; Medus, Meyer, Smith, Jawahir, Miller, Viger, Forstner, Brandt, Nowicki, Salehi, et al., 2009)
Enterobacter spp.	Milk powder	17		2009	Tanzania	(Sánchez-Maldonado, Lee, Farber, & technology, 2018)
S Enteritidis and *S* Typhimurium	Pine nuts	43		2011	United States	(Bedard, Kennedy, Weimer, & Infection, 2014)
E. coli O157:H7	Inshell hazelnuts	17	Product recalled	2011	United States	(Miller et al., 2012)
S. Enteritidis PT 13a	Dried vegetable spice mix	174		2014 – 2015	Sweden	(Sánchez-Maldonado et al., 2018)
E. coli O26	Flour	21		2019	United States	(Vasser et al., 2021)

Table 1.2 Studies on survival kinetics of foodborne pathogens on low-moisture foods under storage.

Commodity	Pathogen	Storage condition	Survival (log CFU/g)	References
Chickpea, sesame, pine nuts, black peppercorns	*L. monocytogenes*	RH: 25, 45, and 75%, 25°C,180 days	4.63 pinenuts > sesame seeds > chickpeas > pepper	Salazar et al., 2019
Peanuts and products	*Salmonella spp.*	28°C, 420 days	2.53 - 3.82	Nascimento et al., 2018
Powdered infant formula	*L. monocytogenes, STEC, S. enterica, C. sakazaki*	5, 22, and 35°C, 365 days	*C. sakazaki* > *Salmonella* > *L. mono* = *STEC* is the desiccation tolerance	Koseki, Nakamura et al. 2015
In-shell Hazelnuts	*STEC*	RH: 40 %, 24°C, 365 days	3	Feng, Muyyarikkandy et al. 2018
Corn flakes, dry shelled pistachios and Chocolate liquor	*L. monocytogenes*	RH: 25 to 81 %, 4°C, 365 days	1.8 to 3.7	Ly, Parreira et al. 2020
In-shell walnuts	*S. enterica*	35 % RH, 4 and 23°C, 1095 days	3 to 5 after 1 to 3 years respectively	Blessington, Theofel et al. 2013
Walnut kernels	*Salmonella*	23°C, 365 days	3	Blessington, Mitcham et al. 2012
Non-fat dry milk	*L. monocytogenes*	4 and 22°C, 365 days	>6	Ballom, Tsai et al. 2020

Table 1.3 Selected list of inactivation studies on low-moisture foods.

Treatment method	Pathogen	Commodity	Treatment conditions	Results (reduction)	Reference
Radio frequency	*S.* Enteritidis	Almonds	40 s	3.7 log CFU/g	(Jeong, Baik, & Kang, 2017)
Convectional heating	*S.* Enteritidis	Almonds	600 s	3.7 log CFU/g	(Jeong et al., 2017)
Thermal treatment	*Salmonella* spp	Cocoa powder	a_w: 0.30, 70-80°C	D-values: 46.2 - 11.5 min	(Tsai et al., 2019)
Thermal treatment	*Salmonella* spp	Cocoa powder	a_w: 0.45, 70-80°C	D-values:31.6–7.0 min	(Tsai et al., 2019)
Thermal treatment	STEC	Wheat flour	MC 13%, 82°C, 5 min	6 log CFU/g	(Daryaei et al., 2020)
Thermal treatment	Shiga toxin *E. coli*	Wheat flour	Moisture level: 8 %, 82°C, 5 min	1.7 log CFU/g	(Daryaei et al., 2020)
Thermal treatment	*L. monocytogenes, Salmonella* spp*., E. faecium*	Powdered infant formula, Peanut butter, Wheat flour	60-90°C	Powder infant formula > peanut butter > wheat flour	(Quinn, Liao, Steele, Jefferies, & Taylor, 2021)
UV-C	*E. coli* O157:H7	Black pepper powder	6 min	1.26 log CFU/g reduction	(M.-J. Park, Kim, Oh, & biotechnology, 2019)
UV-C	*Salmonella* Typhimurium	Black pepper powder	6 min	2.91 log CFU/g reduction	(M.-J. Park, Kim, Oh, et al., 2019)

Table 1.4 Effect of inactivation methods on properties of LMFs

Treatment method	Commodity	Changes in properties	References
UV-C	Wheat flour	• Increased solubility of Osborne proteins • Oxidation and reduction of flour particles • Increased glutenin fraction and decreased albumin fraction	(Kumar, Nayak, Purohit, & Rao, 2021)
LED	Wheat flour	• Bleaching of flour • Increased disulphide linkages • Improved water holding capacity of flour	(L. Du, A. J. Prasad, M. Gänzle, & M. J. F. R. I. Roopesh, 2020)
Heat treatment	Lima bean flour	Retained nutritional composition	(Ogechukwu, Ikechukwu, & Nutrition, 2017)
Heat treatment	Millet flour	Increased pasting viscosity of flour	(Sun, Gong, Li, & Xiong, 2014)
Heat treatment	Sorghum flour	• Improved the flour shelf life • Improved sensory properties as per consumers • Improved physiochemical properties such as bread and cake making	(Marston, Khouryieh, Aramouni, & Technology, 2016; Meera, Bhashyam, Ali, & Technology, 2011)
Heat treatment	Sweet potato flour	• Increased gas retention capacity • Improved dough making properties	(Pérez, Mu, Zhang, Ji, & International, 2017)

CHAPTER II

EFFECT OF STORAGE RELATIVE HUMIDITY ON THE SURVIVAL KINETICS OF *SALMONELLA* SPP., IN DIFFERENT TREE NUT FLOURS

Abstract

The purpose of this study focused on determining the effect of relative humidity (RH) on the survival kinetics of *Salmonella enterica* in tree nut flours during long-term storage. Three types of tree nut (chestnut, hazelnut, and almond) flours were seed inoculated with *Salmonella* (3-strain) to achieve approximately 10^8 log CFU/g and stored at three different RH (25, 45, and 70%) conditions under ambient temperature (21±2°C) storage for up to 120 days. Analysis of samples was done at regular intervals (0, 7, 14, 21, 30, 60, 90, and 120 days) to determine the survivors on both selective and non-selective media. *Salmonella* populations declined by 1.9 x 10^2 – 4.7 x 10^4 CFU/g on the tested flours during the storage period. Compared to RH 25%, 70% observed a significant ($P \leq 0.05$) decrease in *Salmonella* survival. The type of flour showed variable effect on the *Salmonella* survival at tested conditions. For example, after 120 days of storage at 70% RH, reductions were higher on hazelnut flour followed by almond and chestnut flours, respectively. CFU decreases were found to be higher during initial 60 days of 25 and 45% RH storage. At the same time, cell viability decreased most between 60 to 120 d in hazelnut and almond flour at 70% RH.

Keywords: *Salmonella* spp.; Tree nuts; Relative humidity; Survival kinetics; Storage.

Introduction

Low-moisture foods, also called as low water activity foods, include foods that have water activity (A_w) lower than 0.7. Historically, these foods were considered microbiologically safe, but over the past years, these foods have found their association with major foodborne pathogen outbreaks (Gurtler et al., 2014). Nuts and nut products are some of the most important food commodities in this category. Production and consumption of nuts and their processed products have increased many folds over the past two decades because of its economic and nutritional benefits (INDFC, 2012). *Salmonella* is considered as one of the most problematic pathogens of concern in these products. More than 50 recalls of nuts have been issued in the US between 2001 and 2020 due to potential contamination with *Salmonella* (Yada & Harris, 2018). Nuts such as pistachios (Harris et al., 2016), almonds (Bansal, Jones, Abd, Danyluk, & Harris, 2010), peanuts (Calhoun, Post, Warren, Thompson, & Bontempo, 2013), walnuts (T. Blessington, C. G. Theofel, E. J. Mitcham, & L. J. Harris, 2013a), and pecans (Brar, Strawn, & Danyluk, 2016) were found to be contaminated in the recent past.

Pathogen contamination of nuts and processed products can occur either under wet or dry conditions at many stages during harvesting, post-harvest handling, and processing which are either performed manually or mechanically in different parts of the world. Contaminated irrigation water, manure, grazing animal, and wildlife is a potential source of foodborne pathogens for on-farm nut contamination (Mattick, Jørgensen, Legan, Lappin-Scott, & Humphrey, 2000). Several factors affect the survival of *Salmonella*; nut A_w is therefore kept under 0.7 to prevent bacterial and fungal growth. Although *Salmonella* is not reported to grow under low moisture conditions but LMFs have been shown to improve survival LMFs (Beuchat et al., 2013a). *Salmonella* can be introduced in the processing environment and is known to

persist in the processing facility for several months. This increases the chance of cross-contamination to processed products like nut flours which are further used as a raw ingredient for baking and confectionary (Frelka & Harris, 2014). These flours are kept under storage for varying amounts of time. Relative humidity can vary greatly under storage conditions and is known to affect the A_w of foods and therefore affect the survival of foodborne pathogens. (Salazar et al., 2019a) reported increased survival of *Listeria monocytogenes* on pine nuts, chickpeas, black pepper, and sesame seeds stored at 25% RH and lowest at 75% RH. However, no data on *Salmonella* survival in tree nut flours at different relative humidity under ambient storage conditions is available in the literature. Thus, the objective of our present study was to determine the survival kinetics of *Salmonella* spp., on almond, chestnut and, hazelnut flours at RH 25, 45, and 70%, during 120 days storage period.

Materials and Methods

Microbial evaluation of tree nut flours

Almond, chestnut, and hazelnut flours were selected in this study. All these flours were purchased from a commercial supplier (Nuts.com). The approximate composition of selected tree nut flours based on the product label is shown in Table 1. Flours of each type were first inspected for any visual contamination and tested for background microflora. Briefly, 1 g of each flour type in triplicates were transferred to stomacher bags (Whirl-pak®; Nasco™, USA) containing 10 ml of 0.1% buffered peptone water (BPW). The contents inside stomacher bag (Model 400 Seward™, NE, USA) were homogenized, and ten-fold serial dilutions were plated on tryptic soy agar (TSA) followed by incubation at 37°C for 24±2 h. No bacterial growth was evident on TSA from any of the selected flour samples.

Bacterial strains and culture conditions

A three-strain cocktail of *Salmonella* spp., isolated from different sources, was used in this study as recommended by the National Advisory Committee for Microbiological Criteria for Foods (NACMCF). The serovars used include (i) *S*. Enteridis PT 30 (isolated from raw almonds) (ii) *S*. Tennessee K4643 (clinical isolate from peanut butter outbreak) (iii) *S*. Typhimurium (ATCC 14028) (isolated from poultry). All the strains were obtained from the University of Georgia Center for Food Safety culture collections and protocols drawn from standard practices employed in food safety research. Strains were stored at -50°C in tryptic soy broth (TSB) containing 25% glycerol (wt/wt). Aliquots of frozen cultures of each strain were inoculated in 9 ml TSB and passaged twice at 37°C for 24±2 h prior to each experiment. Next, 100 µl of overnight culture was spread on TSA plates with a sterile L-shaped glass rod and bacteria harvested following overnight incubation at 37°C. Briefly, 5 ml sterile 0.1% BPW was delivered on the lawn that had grown on each plate; cells of each strain were then collected separately and pipetted into sterile 15 ml conical tubes (Corning, Corning, USA). Equal volumes of 3 ml from each culture were pooled to form a cocktail mixture of three strains. The cocktail culture was centrifuged at 4000 x *g* for 10 min (Eppendorf™, Hamburg, Germany). The resulting pellet was washed once with BPW and resuspended in 10 mL 0.1% BPW at a concentration of 10^{8-9} CFU/ml. Cell density was adjusted by measuring the absorbance at OD_{600nm} using a UV/VIS spectrophotometer (Biospectrometer, Eppendorf ™, Germany) and confirmed by plating 10-fold serially diluted 100 µl aliquots on TSA plates followed by incubation at 37 ºC for 24 ± 2 h.

Sample preparation and inoculation

A 100 g sample of each almond, chestnut and hazelnut flour were inoculated separately using seed inoculation method to achieve about 10^{8-9} CFU/g. Initially 10 g of flour were transferred into a stomacher bag and inoculated with 1 ml of the mixed bacterial culture; the sealed bag was thoroughly hand massaged for 5 min to evenly distribute the inoculum across the flour particles and prevent clump formation. The 10 g inoculated flour samples were then transferred into the remaining 90 g of the individual flours in a stomacher bag followed by thorough mixing to achieve an even distribution of the *Salmonella* bacteria. Triplicate samples of each flour type (1 g each) were removed for enumeration of baseline *Salmonella* CFU counts. Uninoculated control samples were prepared similarly by adding sterile BPW instead of inoculum.

Storage of inoculated flours

The inoculated samples (97 g each) were divided into three equal portions of approximately 32.2 g, placed into brand new sterile zip-lock bags (Sklar, West Chester, PA) and stored in desiccator chambers (Bel-Art Products, South Wayne, NJ) at 23±2°C and 25, 45 and 70% RH, respectively. The RH levels inside the chambers were maintained using saturated salt solutions of potassium acetate (25%), potassium carbonate (45%), and sodium chloride (70%), respectively. RH and temperature levels inside the desiccator chambers were periodically monitored using data loggers embedded in the chambers as well as cross-checked with a psychrometer with IR thermometer (HD 500, EXTECH, CN). All experiments at each storage conditions were carried out in duplicates.

Microbial enumeration

At each sampling time: 0, 1, 7, 14, 21, 28, 45, 60, 90, 120, 180 days, 1g samples of each flour were collected in triplicates. The collected samples were added to stomacher bags containing 9 ml of 0.1% BPW followed by homogenization in the stomacher for 2 min at 230 rpm. Ten-fold serial dilutions prepared in 0.1% BPW were used for plating on selective Xylose Lysine Deoxycholate (XLD) and non-selective TSA media. Following incubation at 37°C for 24 h and CFUs enumerated. No significant difference in the counts was observed between selective and non-selective media. Thus, XLD data was used for discussion.

Statistical Analysis

All the experiments were performed in duplicates with triplicate 1 g samples collected in each experiment at the tested conditions (Pooled data n=486). The number of cells were transformed to log CFU/g. The survival data was analyzed by: (i) comparing the plate counts between each storage condition (i.e. RH 25, 45, and 70%) and flour type (Almond, Chestnut and Hazelnut), (ii) comparing within the flour type across different RH. In addition, statistical analysis of survival data was performed within the same storage condition across different time points. Analysis of variance (ANOVA) was performed on CFU counts using SPSS™ (Version 25, IBM®). Tukey's honestly significant difference test was used for determination of the mean differences. Significant difference was considered for P value < 0.05.

Results

Water activity of flours during the storage

Changes in the A_w of tree nut flours during storage are shown in Fig 2.1. Initial A_ws of uninoculated almond, chestnut, and hazelnut flours were found to be 0.5589, 0.3718, and 0.5350, respectively. After the inoculation (i.e., Day 0), A_ws of samples increased to 0.6552 (almond

flour), 0.4685 (chestnut flour), and 0.6711 (hazelnut flour). Upon storage in RH controlled chambers, the samples spontaneously equilibrated to a mean A_w of 0.3217, 0.4898, and 0.6211 after 1st day at 25, 45, and 70% RH conditions, respectively. Subsequently, the A_w levels of almond, chestnut, and hazelnut flours stored at 25% RH ranged from 0.303-0.328, 0.287-0.319, and 0.295-0.361, respectively, during the 120 days storage period. While the A_w levels ranged from 0.467-0.499, 0.460-0.498, and 0.429-0.499 at 45% RH and 0.622-0.664, 0.603-0.697, and 0.639-0.704 at 70% RH for almond, chestnut, and hazelnut flours, respectively. Upon inoculation with liquid bacterial suspensions, flour samples were expected to display an A_w increase. During equilibration, all samples experienced slight A_w fluctuation but then stabilized for the remainder of the storage period. Similar changes in A_w have been reported when chickpeas, sesame seeds, pine nuts, and black pepper were stored at different RH conditions (Salazar et al., 2019).

Effect of RH on the survival of Salmonella

Fig. 2.2 shows the survival kinetics of *Salmonella* in tree nut flour at different RH (25, 45, and 70%) storage. The storage RH reflected significant effect ($P \leq 0.05$) on the survival of *Salmonella.* For example, almond flour inoculated with 8.7 x 10^8 CFU/g when stored at 25% RH (Fig 2.2A) gradually decreased to 1.02 x 10^5 CFU/g by 120 days. At RH 45% *Salmonella* cell counts decreased to 1.3 x 10^6 log CFU/g (Fig 2.2A) and at RH 70%, bacteria were recovered from almond flour at 3.4 x 10^4 CFU/g after 120 days storage (Fig 2.2A). This indicates that *Salmonella* viability was favorably impacted at 25 and 45% RH conditions compared to 70%. All bacteria, regardless of RH condition, experienced initial fluctuations, but once they stabilized to set storage conditions, a gradual fall in viability was found. Similar trends in the *Salmonella* reduction were observed in a chestnut (Fig 2.2B) and hazelnut (Fig 2.2C) flour.

Effect of type of flour on the survival of Salmonella

Table 2.1 shows the effect of type of flour on the survival kinetics of *Salmonella* when stored at different RH conditions. When almond flour was stored at 25% RH a significant reduction of 1.53 log CFU/g was observed after 28 days of storage. Under identical storage conditions, a significant reduction of 1.83 log CFU/g after 14 days and 1.6 log CFU/g after 60 days was observed in hazelnut and chestnut flours, respectively. Subsequently, cell counts gradually decreased to 5.01 (almond flour) and 5.4 (chestnut and hazelnut flours) log CFU/g, respectively up to 120 days of storage. However, no significant difference ($P>0.05$) was observed in *Salmonella* cell counts between 60 to 120 days of storage (Table 2.1). These results indicate that flour type significantly impacted survival kinetics of *Salmonella* at least during the first 60 days of storage at 25% RH.

At 45% RH, similar trends in reductions across different flour types were recorded. A significant ($P\leq0.05$) reduction of 2.1, 1.62, and 1.69 log CFU/g was observed on almond, chestnut, and hazelnut flours, respectively, after 60 days of storage. A significant difference ($P\leq0.05$) in *Salmonella* cell counts was found between almond and chestnut and/or hazelnut flours at the end of 120 days storage (Table 2.1). At the same time, no significant difference ($P>0.05$) was found between chestnut and hazelnut flours. At 70% RH, *Salmonella* levels decreased to 3.27 log CFU/g in chestnut flour after 120 days of storage. A significant difference in *Salmonella* cell counts was observed in almond, chestnut, and hazelnut flours stored at 70% RH. *Salmonella* was found to retain improved viability in chestnut (4.88 log CFU/g) followed by hazelnut (4.63 log CFU/g) and almond (4.53 log CFU/g) flours, respectively after 120 days of storage. These findings suggest that *Salmonella* viability is affected differentially when stored in almond, chestnut, or hazelnut flour under otherwise identical RH storage conditions. Similar

findings were observed when wheat flour and skim milk powders were inoculated with *Saccharomyces cerevisiae,* cell counts declined from 6 x 10^8 cells/g to 1.07 x 10^8 and 2.09 x 10^7 cells/g on wheat flour and skim milk powder, respectively (Laroche, Fine, & Gervais, 2005).

Discussion

Factors such as the type of commodity, storage conditions, A_w of samples, and inoculum density has shown to significantly influence *Salmonella* survival. This study indicates that *Salmonella* displayed improved viability at lower RH (25 or 45%) compared to higher RH (70%) conditions after 120-day storage in tree nut flours. Similar trends were *Listeria monocytogenes* populations when tested on pine nuts, chickpeas, sesame seeds, and black peppercorns (Salazar et al., 2019b). *Salmonella* survival improved in chestnut followed by almond and hazelnut flour. Farakos, Pouillot & Keller (2017) reported that *Salmonella* was most tolerant on pine nuts then on pecans followed by hazelnuts, and survival decreased under higher RH storage conditions. They reported the same 1 log decline of *Salmonella* CFUs stored for 24±2 or 9±1 week on hazelnuts at 25°C and 0.37±0.009 or 0.54±0.009 A_w, respectively. *Salmonella* cell counts decreased from 6 to <1 log CFU/g on walnuts stored for 60 days at ambient storage conditions (Blessington et al., 2013b). Experimental results reported here indicate that *Salmonella* viability was higher during the first 60 days compared to the remaining 60 days of storage. This trend was observed in all flours tested regardless of storage RH. Blessington, Theofel et al. (2013) reported that *Salmonella* cell counts in LMFs decreased by 3.6 log CFU/g during the first 1.2 years of storage and then 2 log CFU/g between 1.2 and 3.1 years of storage at ambient conditions. No clear consensus exists on how nutrient composition influences survival of *Salmonella* in LMFs; however, some studies have shown that the presence of fat has a protective effect on *Salmonella* viability. For example, Podolak,

Enache et al. (2010) reported a protective effect of high-fat content of chocolate and potato chips on *Salmonella*. While the same study also mentioned that *Salmonella* was extremely resistant in non-fat dried milk (Podolak et al., 2010). Similarly, high-fat content of peanuts was attributed as one of the factors supporting the survival of *S.* Typhimurium (M. S. Nascimento et al., 2018). While another study showed improved viability of *Salmonella* on peanuts of lower fat content (He et al., 2011). Either way, this study indicates that cell viability declined the least in chestnut compared to almond and hazelnut flour although chestnut flour has the lowest fat composition among the three flours. Therefore, in depth studies are required to shed more light on the effect of nutrient composition on the survival characteristics of *Salmonella* on LMFs.

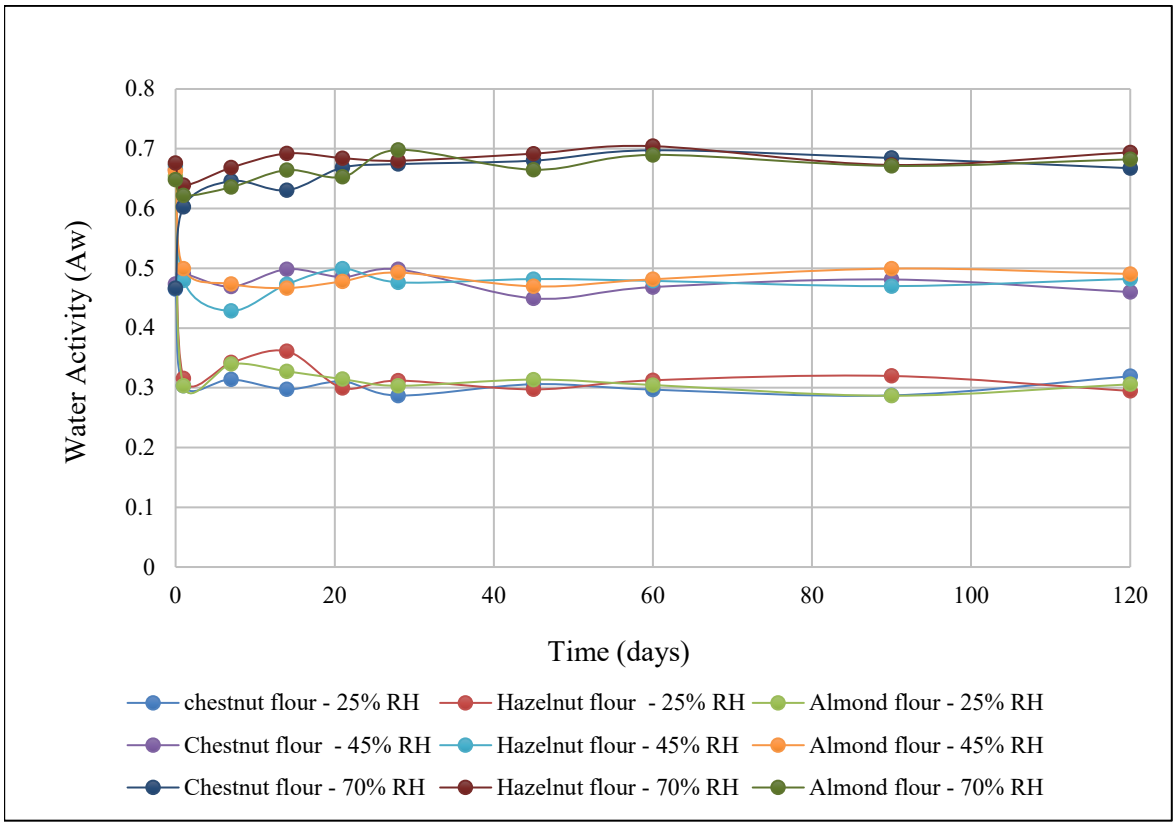
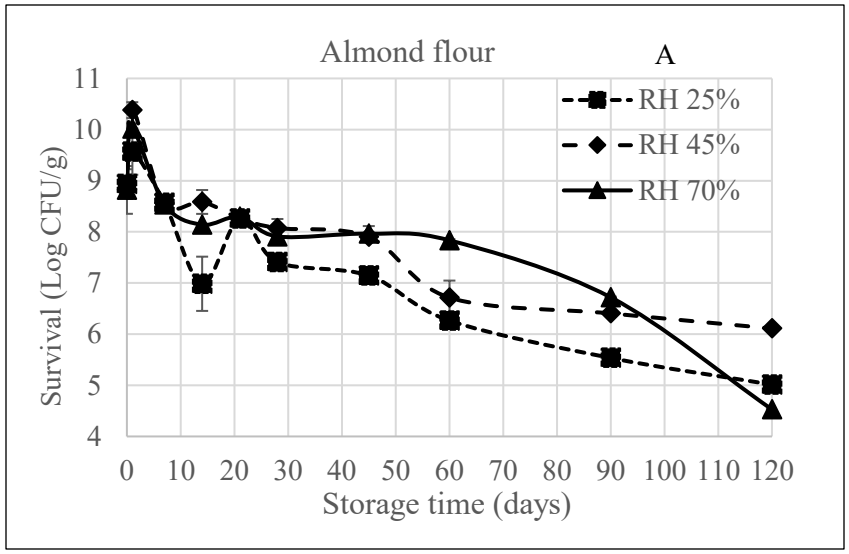
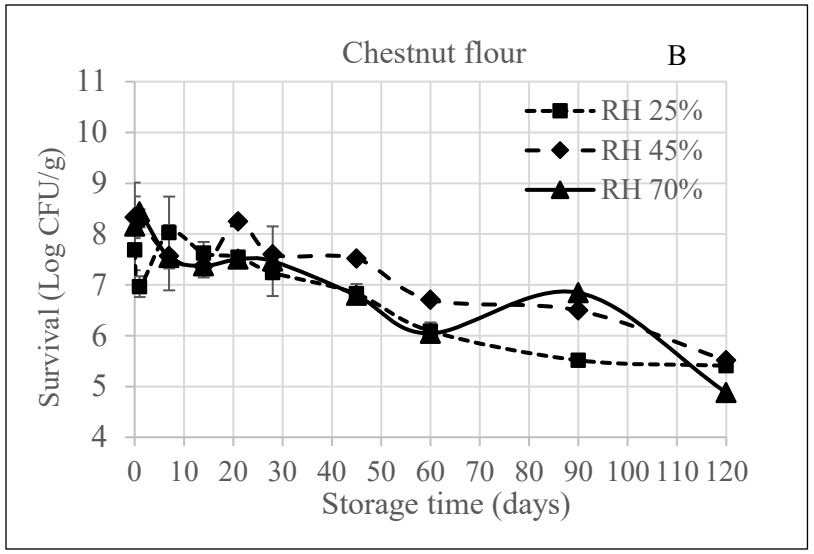


Fig 2.1. Changes in A_w of different tree nut flours when stored at different RH conditions.





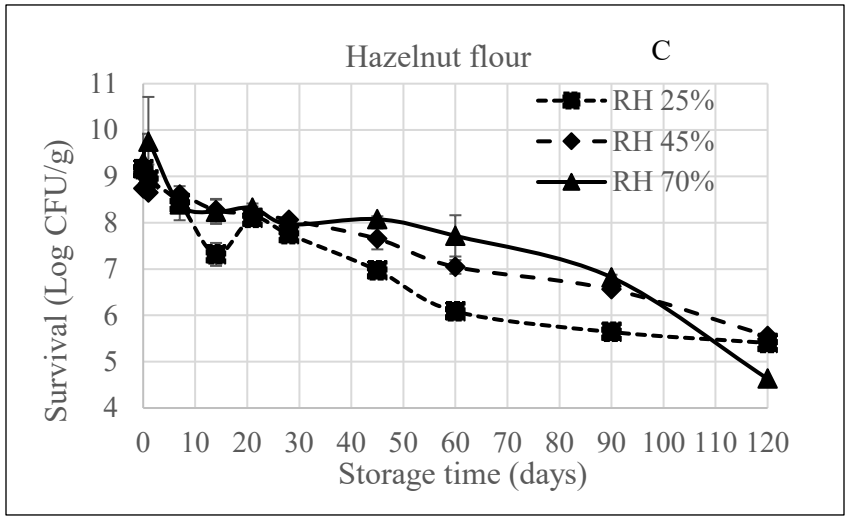


Fig 2.2. Survival of *Salmonella* spp., on (A) almond, (B) chestnut and (C) hazelnut flours at 25, 45 and 70% RH, respectively

Table 2.1 Comparison of survival kinetics of *Salmonella* spp., across different variables

		Reduction of *Salmonella* spp. (log CFU/g) during storage between time 0 and 120 days									
Type of flour	RH (%)	0	1	7	14	21	28	45	60	90	120
Chestnut	25	7.69 ± 0.73^{aAX}	6.97 ± 0.21^{abBY}	8.03 ± 0.71^{aAX}	7.63 ± 0.22^{aAX}	7.55 ± 0.05^{aBY}	7.24 ± 0.04^{abAY}	6.83 ± 0.19^{abcBX}	6.09 ± 0.17^{bcdBX}	5.52 ± 0.08^{cdCX}	5.41 ± 0.00^{dBX}
	45	8.33 ± 0.41^{aAX}	8.27 ± 0.13^{aAY}	7.57 ± 0.08^{bAY}	7.37 ± 0.22^{bAY}	8.25 ± 0.04^{aAX}	7.60 ± 0.07^{bAY}	7.52 ± 0.04^{bAX}	6.71 ± 0.02^{cAX}	6.50 ± 0.05^{cBX}	5.51 ± 0.03^{dAY}
	70	8.15 ± 0.86^{abAX}	8.44 ± 0.05^{aAX}	7.54 ± 0.64^{abcAX}	7.38 ± 0.13^{abcAY}	7.51 ± 0.16^{abcBY}	7.47 ± 0.69^{abcAX}	6.79 ± 0.10^{bcBY}	6.05 ± 0.02^{cdBY}	6.85 ± 0.01^{abcAX}	4.88 ± 0.02^{dCX}
Hazelnut	25	9.15 ± 0.44^{aAX}	8.93 ± 0.33^{abAX}	8.42 ± 0.37^{abcAX}	7.32 ± 0.25^{deAX}	8.10 ± 0.07^{bcdAX}	7.75 ± 0.03^{cdeBX}	6.97 ± 0.01^{efBX}	6.09 ± 0.18^{fgBX}	5.64 ± 0.03^{gCX}	5.40 ± 0.00^{gBX}
	45	8.74 ± 0.08^{aAX}	8.65 ± 0.01^{aAY}	8.61 ± 0.02^{abAX}	8.27 ± 0.25^{abAXY}	8.2 ± 0.22^{abcAX}	8.06 ± 0.04^{bcAXY}	7.65 ± 0.23^{cAX}	7.05 ± 0.15^{dABX}	6.56 ± 0.01^{dBX}	5.55 ± 0.00^{eAY}
	70	9.31 ± 0.62^{abAX}	9.75 ± 0.97^{aAX}	8.4 ± 0.01^{abcAX}	8.24 ± 0.26^{abcAX}	8.32 ± 0.03^{abcAX}	7.97 ± 0.06^{bcAX}	8.08 ± 0.06^{bcAX}	7.72 ± 0.06^{cAX}	6.82 ± 0.06^{cAX}	4.63 ± 0.01^{dCY}
Almond	25	8.94 ± 0.29^{abAX}	9.57 ± 0.45^{aAX}	8.57 ± 0.08^{cAX}	6.99 ± 0.53^{deBX}	8.26 ± 0.01^{bcAX}	7.41 ± 0.17^{cdBXY}	7.15 ± 0.06^{deBX}	6.27 ± 0.02^{efBX}	5.54 ± 0.02^{fgCX}	5.01 ± 0.01^{fgBY}
	45	8.82 ± 0.03^{bAX}	10.38 ± 0.16^{aAX}	8.56 ± 0.01^{bcAX}	8.59 ± 0.23^{bcAX}	8.26 ± 0.03^{bcdAX}	8.08 ± 0.18^{cdAX}	7.9 ± 0.01^{dAX}	6.72 ± 0.33^{eBX}	6.41 ± 0.05^{efBX}	6.11 ± 0.01^{fAX}
	70	8.82 ± 0.47^{bAX}	10.02 ± 0.11^{aAX}	8.54 ± 0.02^{bcAX}	8.14 ± 0.01^{cdABX}	8.3 ± 0.08^{bcdAX}	7.91 ± 0.00^{cdABX}	7.97 ± 0.15^{cdAX}	7.84 ± 0.02^{dAX}	6.72 ± 0.01^{eAX}	4.53 ± 0.01^{fCZ}

Lower-case letters a, b, c, d, e, f, and g represent significant differences between *Salmonella* CFUs within same RH and flour type within any given row.
Upper-case letters A, B, C represent significant difference between *Salmonella* CFUs across 25, 45 and 70% RH conditions within the same flour at each time point.
Letters X, Y, Z represent a significant difference in *Salmonella* CFUs among different flours stored at same RH conditions at each time point.

CHAPTER III

THERMAL AND UV-C INACTIVATION KINETICS OF *SALMONELLA* AND *E. COLI* O157:H7 IN PEANUT FLOUR

Abstract

LMF such as peanuts and peanut products have been implicated in several foodborne disease outbreaks. This study aimed at evaluating the efficacy of hot water and UV-C treatment as thermal and non-thermal methods of *Salmonella* spp. and *E. coli* O157:H7 inactivation in peanut flour. Inoculated samples were first equilibrated at 0.5 A_w and then subjected to hot water treatment at temperatures 60, 70, and 80°C for up to 30 min and UV-C treatment at 25 mW/cm^2 for up to 60 min. CFU counts of *Salmonella* spp. and *E. coli* O157:H7 declined as both thermal and UV-C treatment was increased. *E. coli* O157:H7 was observed to be more sensitive towards thermal treatment than *Salmonella* spp. However, the opposite trend was observed for UV-C treatment. Inactivation of *Salmonella* spp., and *E. coli* O157:H7 in peanut flour was plotted using the Weibull log-linear model due to the decrease in inactivation rate resulting from increasing treatment time. The findings of this study suggest that UV-C may be a viable companion treatment with other risk mitigation strategies against *Salmonella* and *E. coli* O157:H7 contamination in peanut flour. Keywords: Thermal treatment, UVC, Peanut flour, Kinetics, *Salmonella*, *E. coli* O157:H7.

Introduction

Peanuts and their products are rich source of valuable nutrients such as vitamin E,

magnesium, phosphorus and copper and are known to confer various health benefits such towards the reduction of coronary heart diseases and cancer (Griel, Eissenstat, Juturu, Hsieh, & Kris-Etherton, 2004; Pinkas, Battista, & Morille-Hinds, 2009). World peanut production increased by 22.8 million metric tons since the 1970s indicating an increasing demand and consumption of peanut and its products (Fletcher & Shi, 2016). Peanut products fall under the category of low water activity foods (A_w<0.70). Several LMF products such as milk powder, powdered spices, powdered infant formula, peanut butter, chocolate and cereals are emerging as the vehicle for major foodborne pathogens among which *Salmonella* and *E. coli* are of significant concern (CDC, 1993, 2007b, 2016, 2017, 2019; Chang et al., 2013). *Salmonella* has been implicated in several peanut product related outbreaks in the past two decades (CDC, 2007b; Kirk, Little, Lem, Fyfe, Genobile, Tan, Threlfall, Paccagnella, Lightfoot, Lyi, et al., 2004). In 2008, over 500 illnesses, several hospitalized and eight deaths were reported due to peanut product ingestion contaminated with *Salmonella Typhimurium* (Medus, Meyer, Smith, Jawahir, Miller, Viger, Forstner, Brandt, Nowicki, & Salehi, 2009). In 2014, another multistate outbreak of *Salmonella* led to six infections and one hospitalization due to consumption of contaminated peanut product which led to a peanut product recall from the market (Control & Prevention, 2014). Studies indicate that contamination of peanut and its products can occur at several steps during its supply chain. *Salmonella* and *E. coli* contamination was found in nine and seven peanut samples at production and processing stages respectively (M. Nascimento et al., 2018).

Low-water activity has been shown to promote the survival of foodborne pathogens (Beuchat et al., 2013a); for example, *Salmonella* reported survival for more than a year under desiccated conditions in peanut butter (Kataoka et al., 2014; Nummer, Shrestha, & Smith, 2012).

Peanut flour is another processed form of peanuts that is either consumed raw or used as a resource for the manufacture of various confectionery products. Processing of peanuts to end consumer goods can be subjected to cross-contamination, inappropriate roasting or post-processing contamination (Control & Prevention, 2007; Roberts et al., 2005). The US FDA has raised awareness to the public as recently as 2019 that consumption of raw flour and its products is unsafe due to potential contamination with *Salmonella* and harmful *E. coli* (Food & Administration, 2019). (Rose, Bianchini, Martinez, & Flores, 2012) also reported that consumers are at serious risk due to cross-contamination and handling habits of contaminated LMFs. Therefore, to ensure microbial safety of peanut flour either in raw or processed form, it is necessary to ensure the implementation of appropriate treatment methods to curtail the risk of *Salmonella* and toxigenic *E. coli* contamination.

Several treatment interventions are commonly used for this purpose, most notably thermal treatment of powdered foods. However, the efficacy of these treatments while maintaining the quality of food intact is a challenge. A need has emerged for alternative and effective interventions to ensure the safety and quality of LMF (Komitopoulou & Peñaloza, 2009). Exposure of food to Ultraviolet (UV) type C, a high-energy, short-wavelength form of ultraviolet radiation, has shown promise to reduce foodborne pathogens risk on various food matrices. UV-C irradiation gives rise to pyrimidine dimers in DNA upon exposure, therefore, hampering cell viability (Dai, Vrahas, Murray, & Hamblin, 2012). Whereas the exposure of prokaryotic cells to heat or high temperatures results in degradation of cytoplasmic components and cell wall, thereby killing the bacteria and inhibiting growth (Mitsuzawa, Deguchi, & Horikoshi, 2006). Although these methods have been employed to decontaminate some LMFs from various foodborne pathogens, their efficacy depends upon several factors such as surface

characteristics, nature of food products, and components of treatment, including energy dose and treatment time (L. Du, A. J. Prasad, M. Gänzle, & M. Roopesh, 2020). However, few reports describe the potential application of UV-C and the thermal inactivation of *Salmonella* and *E. coli* on LMFs. Due to all aforementioned factors, the potential of UV-C and hot water treatment as inactivation methods of specific LMFs is worth investigating. Thus, the aim of the present study was to determine the efficacy of UV-C as a non-thermal method and hot-water treatment as a thermal method for decontamination of *Salmonella* and *E. coli* in peanut flour and determine pathogen survival kinetics.

Materials and Methods

Selection of peanut flour

Peanut flour obtained from a commercial supplier (Nuts.com) was inspected for any visual contamination and tested for background microflora. Briefly, triplicate samples of 1 g of each were separately suspended in 0.1% BPW into stomacher bags (Whirl-pak®; Nasco™, USA) followed by thorough homogenization using a mechanical stomacher (Model 400 Seward™, NE, USA). Ten-fold serial dilutions were plated on TSA followed by incubation at 37°C for 24±2 h. No CFUs were recovered, indicating that the peanut flour used in the study was free from any background microflora.

Bacterial strains and inoculum preparation

A cocktail of three strains of *Salmonella* and *E. coli* O157:H7 isolated from different sources was used in this study. The serovars used in this study include (i) *S*. Enteritidis PT 30 (isolated from raw almonds) (ii) *S*. Tennessee K4643 (clinical isolate from peanut butter outbreak) (iii) *S*. Typhimurium (ATCC 14028) (isolated from poultry); (iv) *E. coli* O157:H7 (NCTC 12900), (v) *E. coli* O157:H7 (ATCC 35150; Human isolate), and (vi) *E. coli* O157:H7

(EO122; cattle isolate). All the strains were obtained from the University of Georgia Center for Food Safety culture collections and protocols drawn from standard practices employed in food safety research. All the strains were stored at -50°C in TSB containing 25% glycerol (wt/wt). Frozen cultures of each strain were inoculated into 9 ml TSB with 0.6% yeast extract and passaged twice at 37°C for 24±2 h prior to each experiment. A 100 µl inoculum from an overnight culture was spread on tryptic soy agar with yeast extract (TSAYE) using a sterile L-shaped glass rod. Cells were harvested upon overnight incubation at 37°C for 24±2 h by transferring 5 ml of sterile 0.1% BPW on the bacterial lawn that had grown on each plate. Cells of each strain were suspended and transferred with a micropipette into sterile 15 ml conical tubes (Corning, Corning, USA). Equal volumes of each strain (3 ml) were pooled to produce a cocktail mixture separately for either *Salmonella* and *E. coli* O157:H7 strains. Cocktail cultures were centrifuged at 4000 x *g* for 10 min (Eppendorf™, Hamburg, Germany) and pellets washed once with 9 ml 0.1% BPW followed by resuspension in 9 ml 0.1% BPW resulting in the cell density of 10^{8-9} CFU/ml. The strain mixture was serially diluted and plated on TSAYE to determine the initial cell concentration (CFU/ml).

Sample preparation and inoculation of sample

A 100 g sample of peanut flour was inoculated with *Salmonella* using the seed inoculation approach. Initially, 10 g flour samples were placed into a stomacher bag (Whirl-pak®; Nasco™, USA) and inoculated with 1 ml of freshly prepared three-strain cocktails of *Salmonella* or *E. coli* O157:H7. The inoculated samples following inoculation in the sealed bags were thoroughly hand massaged for 5 min to evenly distribute the inoculum and prevent clump formation. This inoculated 10 g flour sample was then transferred into the remaining 90 g flour followed by thorough mixing to achieve homogenous distribution of bacterial cells

throughout the flour samples. Subsequently, 1g of inoculated samples in triplicates was enumerated for initial *Salmonella* or *E. coli* O157:H7 cell counts yielding a mean of 9.78±0.34 log CFU/g. Control samples were inoculated similarly using 0.1% BPW alone.

Equilibration of samples at selected A_w conditions

All samples were equilibrated for 48 h in an environmental chamber (Thermo Fisher, OH, USA) and maintained at 50% RH and 23°C. Following equilibration, the mean A_w values of the samples were found to be 0.51±0.01 prior to subjecting to thermal and non-thermal treatments mentioned below.

Thermal Inactivation

Thermal inactivation was carried out by subjecting the samples to hot-water treatment as follows. Hot water baths (Thermo Scientific, Waltham, MA, USA) were maintained at either 62 or 72 and/or 82°C to reach the target temperatures of 60, 70, and 80°C, respectively, inside the bagged samples. Optimization experiments also indicated that 2 – 3.4 min are required to reach target temperatures inside the product and a cooling time of 0.5 - 1 min in the ice bath returns the treated samples to ambient temperature. Next, Whirl-Pak™ bags containing 1g of sample each were taped to a metal rod and bulldog clamps attached to the bottom to keep the bags submerged (Fig 3.1) into the water bath maintained at the set temperature. A K-type thermocouple was inserted into an uninoculated control sample's cold or thermal point (i.e., the geometric center of food or container) to monitor the temperature during the treatments. Triplicate samples were immersed into the water at the specified temperatures for the duration of 0, 2.5, 5, 7.5, 10-, 20-, and 30-min treatment times. Following thermal treatment, the bags at each sampling time were

immediately suspended into ice-water bath for bringing temperature to ambient conditions before proceeding to microbial analysis.

UV-C Inactivation

A custom-built UV-C cabinet equipped with a 55W UVC tube (64.77 x 12.95 x 6.9 cm^3) (Model 201014, GermAwayUV, Delray Beach, FL, USA) and aluminum foil-covered cabinet walls was used UV-C inactivation experiments (Fig 3.2). UV-C lamp was switched on 10 minutes prior to the treatment for emission to stabilize. Inoculated samples weighing 1g after equilibration to target A_w (~0.5) were transferred into sterile Whirl-Pak™ bags. The samples inside the bag were flattened using a roller to produce a ~0.1 cm product layer. These samples were then placed at an 8 cm distance from the UV-C lamp on a transparent thin glass platform producing a 25 mW/cm^2 intensity at the sample surface as measured with a photometer (PM100D, Thor Labs, Bergkirchen, Germany). Samples were then subjected to UV-C exposure at 254 nm for 0, 5, 15, 30 and, 60 min. Changes in temperature on the sample surface were recorded using an IR thermometer (Model HD 500, Extech Instruments, Nashua, NH, USA).

Microbial enumeration

Following treatments, bags containing samples were aseptically opened, and 1g each of treated samples were suspended in 9 ml of 0.1% BPW. Homogenization of the suspended samples were carried out at 230 rpm for 2 min using the stomacher (Model 400, Seward™, NE, USA). Ten-fold serial dilutions of homogenized samples were developed in 0.1% BPW and spread plated on TSAYE as described before. Colonies were enumerated and transformed into Log CFU/g following incubation at 37°C for 24 h, and used for data analysis.

Determination of inactivation kinetics

The inactivation kinetics of *Salmonella* and *E. coli* O157:H7 was described using Weibull model on peanut flour (Peleg, biosystems, & inactivation, 2006)

$$\ln (N/N_0) = - (t/\alpha)^{\beta} \quad (1)$$

$$t_d = \alpha(-\ln(10^{-d})^{(1/\beta)}) \quad (2)$$

$$\log \alpha/\alpha^* = (T^* - T)/z_T \quad (3)$$

Equation (1) represents the various parameters associated with Weibull model. N and N_0 are the CFUs of bacteria at treatment time t and at 0 min, respectively, while α and β are the first ln reduction time and shape factor, respectively. The inactivation curve slope is determined by scale parameter, α, and the shape of the curve is determined by β, which is also known as the shape parameter, $\beta > 1$ refers to the convexity of the curve, while $\beta < 1$ refers to the concavity of the curve. These model parameters were fitted to the experimental inactivation data using the Microsoft Office Excel Solver function at all the treatment conditions, and predictions for one log reduction time was made using equation (2), where parameter d refers to the number of decimal reductions (Gautam, Govindan, Gänzle, & Roopesh, 2020; van Boekel, 2002). The influence of treatment temperature was determined using secondary models, and Z_T was determined using equation (3) and refers to the temperature change needed to reduce the α value of bacterial by 90%, whereas α* refers to the first ln reduction time at the temperature chosen as

reference (T*) (Gautam et al., 2020; Mafart, Couvert, Gaillard, & Leguérinel, 2002). The reference temperature for this study was chosen to be 80ºC.

Statistical Analysis

All the experiments were conducted in two replicates with duplicate samples collected in each experiment at the tested treatment conditions (Pooled data n=384). The number of cells were transformed to log CFU/g. The inactivation data was analyzed by: (i) comparing the plate counts between each treatment time across different treatment conditions, (ii) comparing within each treatment condition across different treatment time. Data analysis was carried out by the analysis of variance (ANOVA) procedure using SPSS^{TM} (Version 25, IBM®). Tukey's least significant difference test was used for determining the mean differences. Differences among mean values were considered significant at $p < 0.05$. Data was fitted into models using Microsoft excel solver function and best model was selected on the basis of R^2 values.

Results and discussion

Thermal Inactivation

***Salmonella* spp.**

Fig. 3.3 shows the reduction of *Salmonella* CFUs in peanut flour at different treatment temperatures. Results indicate that treatment time and temperature showed a significant effect ($p \leq 0.05$) on the survival of *Salmonella*. When subjected to hot-water treatment at 60°C, colony counts decreased by 2.7 log CFU/g after 2.5 min (Fig 3.3). However, no significant difference $(p > 0.05)$ was observed when treatment time was extended up to 30 min. *Salmonella* colony counts declined by 2.51 log CFU/g after 30 min of treatment time. Treatment temperatures of 70°C showed a similar reduction trend. *Salmonella* cell counts declined significantly ($p \leq 0.05$) by 2.23 log CFU/g within the first 2.5 min of treatment. A cumulative decrease in cell counts of

2.89 log CFU/g was observed after 30 min of treatment (Fig 3.3). At a treatment temperature of 80°C *Salmonella* colony counts declined by 3.23 log CFU/g after 30 min of treatment time (Fig 3.3) with the largest drop (2.7 log CFU/g) in viability occurring within 2.5 minutes of treatment as observed at other temperatures tested. Results of this study show that *Salmonella* viability declines ranging between 2 – 3 log CFU/g are achievable within 2.5 min of hot water treatment at either 60, 70 or 80ºC. However, >3 log reduction was accomplished only upon 30 minutes of treatment at 80ºC. A similar trend was reported by (Forghani et al., 2019a) when wheat flour inoculated with *Salmonella* was subjected to 55, 60, 65, and 70 ºC treatment; viability counts remained nearly unaltered (< 0.5 log) after an initial sharp decrease of 0.5 - 1.5 log. Another study (Kharel, Yemmireddy, Graham, Prinyawiwatkul, & Adhikari, 2018) on *Salmonella* inoculated pecans subjected to hot-water treatment reported a 2 log CFU reduction at 70°C and a greater than 3 log decrease at 80°C after 30 min.

***E. coli* O157:H7**

The effect of hot-water treatment on *E. coli* O157:H7 CFU recovery is shown in Fig 3.4. Treatment temperature and time had a significant ($p \leq 0.05$) effect on *E. coli* O157:H7 viability. Peanut flour inoculated with $10^{8\text{-}9}$ *E. coli* O157:H7 CFU/g was subjected to hot water treatment at 60°C, displayed a viability decline of 1.37 log CFU/g ($P \leq 0.05$) after 2.5 min of treatment (3.4). Treatment times extended to 7.5, 10, and 30 min showed a significant ($p \leq 0.05$) cell count reduction of 1.87, 2.39, and 2.60 log CFU/g, respectively. At a treatment temperature of 70°C *E. coli* O157:H7 viability decreased significantly after 2.5, 10, and 20 minutes and a cumulative decline of 2.65 log CFU/g after 30 min (Fig 3.4). At treatment temperature 80°C, the highest reductions of *E. coli* O157:H7 cell counts were recorded at 3.23 log CFU/g after 30 min of treatment (Fig 3.4). Similar to *Salmonella*, the rate of decline of *E. coli* O157:H7 on peanut flour

was higher during the initial treatment phase regardless of temperature. These results are substantiated by a study reporting multiple EHEC strains belonging to the serogroups O45, O121, and O145 that showed a similar initial reduction in viability trends when exposed to heat (Forghani et al., 2019a). Decreases in *Salmonella* and *E. coli* O157:H7 viability as shown in this study may not completely align with other studies as microbial inactivation depends on several factors such as ingredient composition and A_w conditions, which either independently or synergistically determine bacterial heat tolerance (Beuchat et al., 2013b; Burnett, Gehm, Weissinger, & Beuchat, 2000; E. J. Park, Oh, & Kang, 2008; Shachar & Yaron, 2006).

UV-C inactivation of *Salmonella* and *E. coli* O157:H7

The effect of UV-C treatment on *Salmonella* and *E. coli* in peanut flour is shown in Fig 3.5. The treatment time showed a significant ($p \leq 0.05$) effect on *Salmonella*. *Salmonella* with an initial concentration of 9.79 log CFU/g on peanut flour significantly ($p \leq 0.05$) reduced by 1.14, 2.22, and 2.59 log CFU/g after 5, 15, and 30 min of treatment time, respectively. Further increasing the treatment time from 30 to 60 min showed statistically no significant ($p > 0.05$) difference in the reduction. *Salmonella* population declined sharply during the first 5 min of treatment, and the rate of *Salmonella* inactivation reduced during the later stage of the treatment as *Salmonella* population declined only 1.08 log units between 5 and 15 min of treatment. The results of our study are in agreement with other literature-reported studies. For example, black pepper powder, when subjected to UV-C treatment, showed a 2.91 log reduction of *S. enterica* serovar Typhimurium in the first 6 min and only 0.88 log units reduction between 6 and 42 min. (M.-J. Park, Kim, Oh, et al., 2019). Similarly, UV-C inactivation of *S. enterica* on coconut flakes showed 1.55 log reduction after the first minute of exposure, while comparable reductions were observed between 1 and 40 min of treatment time (Gabriel, Tongco, & Barnes Jr, 2017).

Condón-Abanto, Condón et al. (2016) reported that UV-C decontamination of *Salmonella* Typhimurium on flour powder was also non-linear, with quick inactivation during the first minute followed by a tail.

The initial levels of *E. coli* O157:H7 on peanut flour were 9.03, which significantly ($p \leq 0.05$) reduced by 0.9 log units after the first 5 min of UV-C treatment. Increasing the treatment time to 15 min showed a significant reduction of 1.93 log CFU/g. was observed after 15 min treatment. Further increasing the treatment time to 30 and 60 min showed no significant change ($p > 0.05$) in the reduction. An overall *E. coli* O157:H7 reduction of 2.04 log CFU/g was observed after 60 min UV-C treatment of peanut flour. While similar treatment conditions resulted in 2.96 log reduction of *Salmonella*. It suggests that *E. coli* O157:H7 seems to be more resistant to UV-C treatment compared to *Salmonella* at similar treatment conditions. These results are similar to the effect of UV-C on *E. coli* O157:H7 on black pepper powder, which shows that *Salmonella* reduced 1.65 and 1.51 log units more than *E. coli* O157:H7 after 6 and 42 min of treatment with UV-C (M.-J. Park, Kim, & Oh, 2019).

Modeling the inactivation kinetics of *Salmonella* and *E. coli* O157:H7

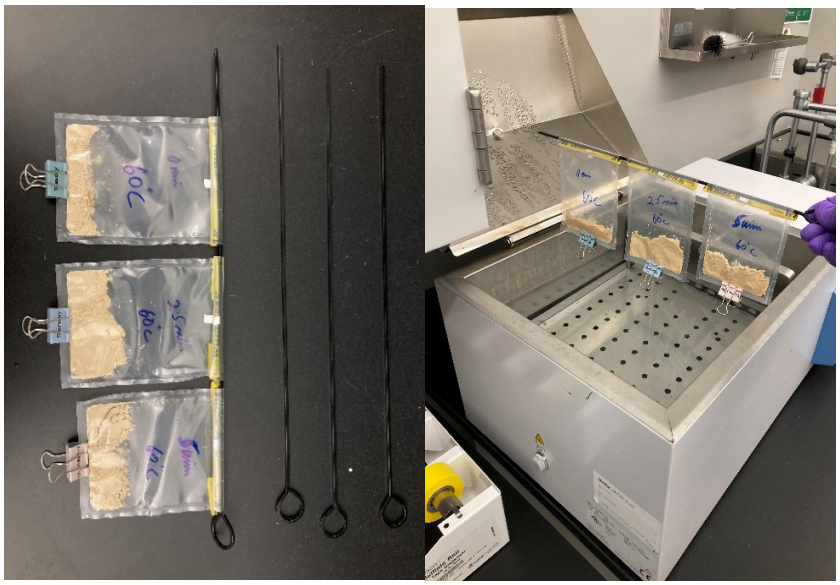
The inactivation kinetics of *Salmonella* and *E. coli* O157:H7 showed non-linear trends for the treatment conditions. The linear regression model was used to fit inactivation data but resulted in a lower R^2 value which represents the closeness of observed data to the fitted regression line. Therefore, inactivation data were fitted using the Weibull model due to this non-linear trend. The Weibull parameters β were found to be < 1 for all the treatment conditions. The β < 1 refers to the declining inactivation rate with increasing treatment time (Bevilacqua, Speranza, Sinigaglia, & Corbo, 2015; Gautam et al., 2020). The Weibull model prediction curves are compared with experimental data as shown in Figs 3.3 & 3.4. The time required for 1 log

reduction was predicted for *E. coli* O157:H7 and *Salmonella* using Weibull model. Predicted values showed that the time required to obtain one log unit reduction is higher at lower treatment temperature regardless of bacterial pathogen (Table 3.1). A similar trend was found in a study on thermal inactivation of *Salmonella* on both the pet food pellet and black pepper powder, where Weibull model predictions for reduction times were higher for lower-treatment temperatures (Gautam et al., 2020). The required time for one log reduction in *Salmonella* viability was 24.18 min at 60ºC. Increasing the temperature from 60 to 70ºC reduced the time required for one log reduction (D-value) to 19.42 min. However, at a temperature of 80°C, the inactivation time decreased to 6.47 min. The predicted one log reduction trend by the Weibull model was similar for *E. coli* O157:H7 and *Salmonella*. However, the required time for one log viability reduction of *E. coli* O157:H7 was comparatively lower than for *Salmonella* at 60, 70, and 80ºC. The predicted time required to achieve one log reduction in *E. coli* O157:H7 CFU/g was 19.42, 13.29, and 4.43 min at 60, 70, and 80°C, respectively. This indicates that *E. coli* O157:H7 is predicted to be more sensitive than *Salmonella* to hot-water treatment. Whereas UV-C inactivation kinetics show that the time required to achieve one log reduction in viability of *Salmonella* and *E. coli* O157:H7 are 16.46 and 18.36 min, respectively, indicating that *Salmonella* is more sensitive than *E. coli* O157:H7 as more time is required to achieve similar drops in cell counts. Comparison of predicted time required to achieve one log CFU/g reduction of tested pathogens shows that UV-C treatment performs equally well if not better than hot water treatment at 60 and/or 70°C for 30 min. Secondary models were used to calculate the thermal resistance coefficient (z_T values expressed in °C) for *Salmonella* and *E. coli* O157:H7 (Table 3.1). The R^2 values observed are close to or greater than 0.9 for Weibull model, indicating an appropriate fit. Log linear relationship was followed between one log reduction times and

treatment temperatures for *Salmonella* and *E. coli* O157:H7 on peanut flour (Fig 3.6). The observed trend was similar to that reported in another study where 3 log and 5 log reduction times of *Salmonella* displayed log linear relationships with inactivation temperatures (Gautam et al., 2020). This information is vital for making informed decisions on choosing effective and efficient intervention methods to mitigate the risk of tested pathogens and maintaining the quality characteristics of treated products at the same time. Thermal treatments at higher temperatures, in general, denature proteins and affect the quality of products. UV-C treatment, in contrast, is presumed to be less damaging to the protein structure of the product, especially at lower treatment times. However, the effect of these treatments on the quality characteristics of the tested product is beyond the scope of the present study. Follow-up studies should focus on quality changes on the products when subjected to treatment while ensuring microbial inactivation.

Conclusions

The results of this study insinuate that UV-C treatment performs equally well if not better compared to hot-water treatment. Hot water treatment reduced bacterial load by an average of 2.95 log CFU/g while UV-C resulted in an average 2.50 log CFU/g reduction. Treatment temperature and time or UV-C dosage showed a significant effect in the inactivation kinetics of tested pathogens. The Weibull model predicted the thermal and UV-C inactivation kinetics of tested pathogens on peanut flour. Follow-up studies should focus on the effect of these treatments when pathogens and food commodities are equilibrated at different A_w levels and on the effect of these treatments on food quality and consumer acceptability.



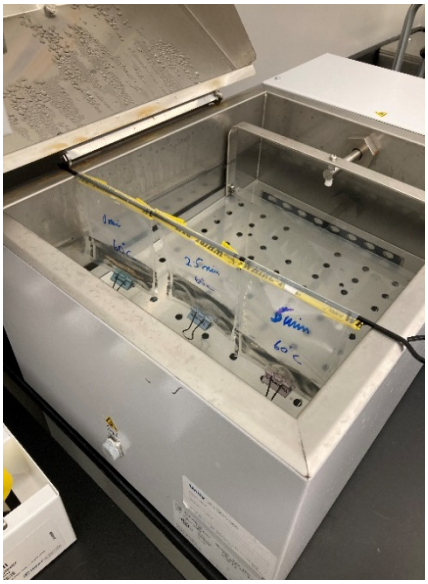


Fig 3.1 Thermal treatment experimental set-up

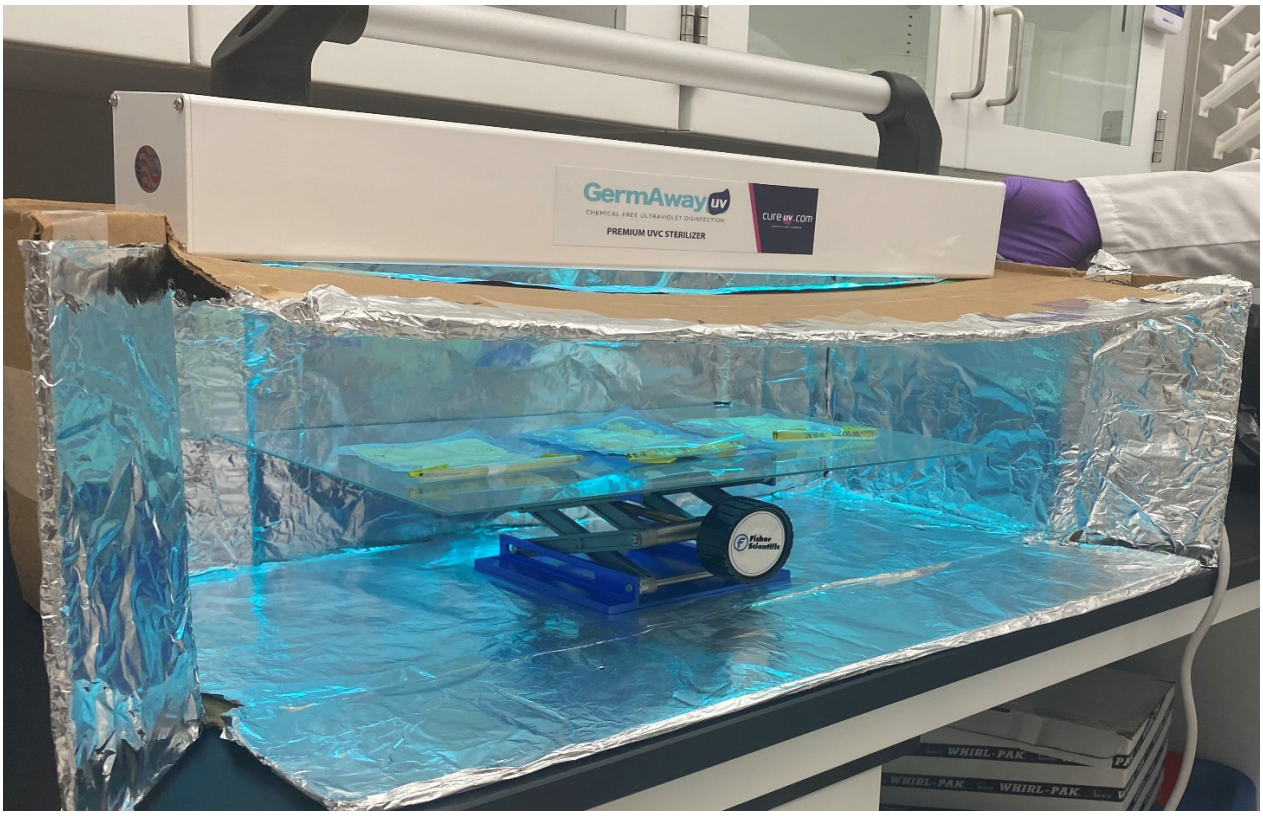


Fig 3.2 UV-C treatment experimental set-up using custom-built UV-C chamber

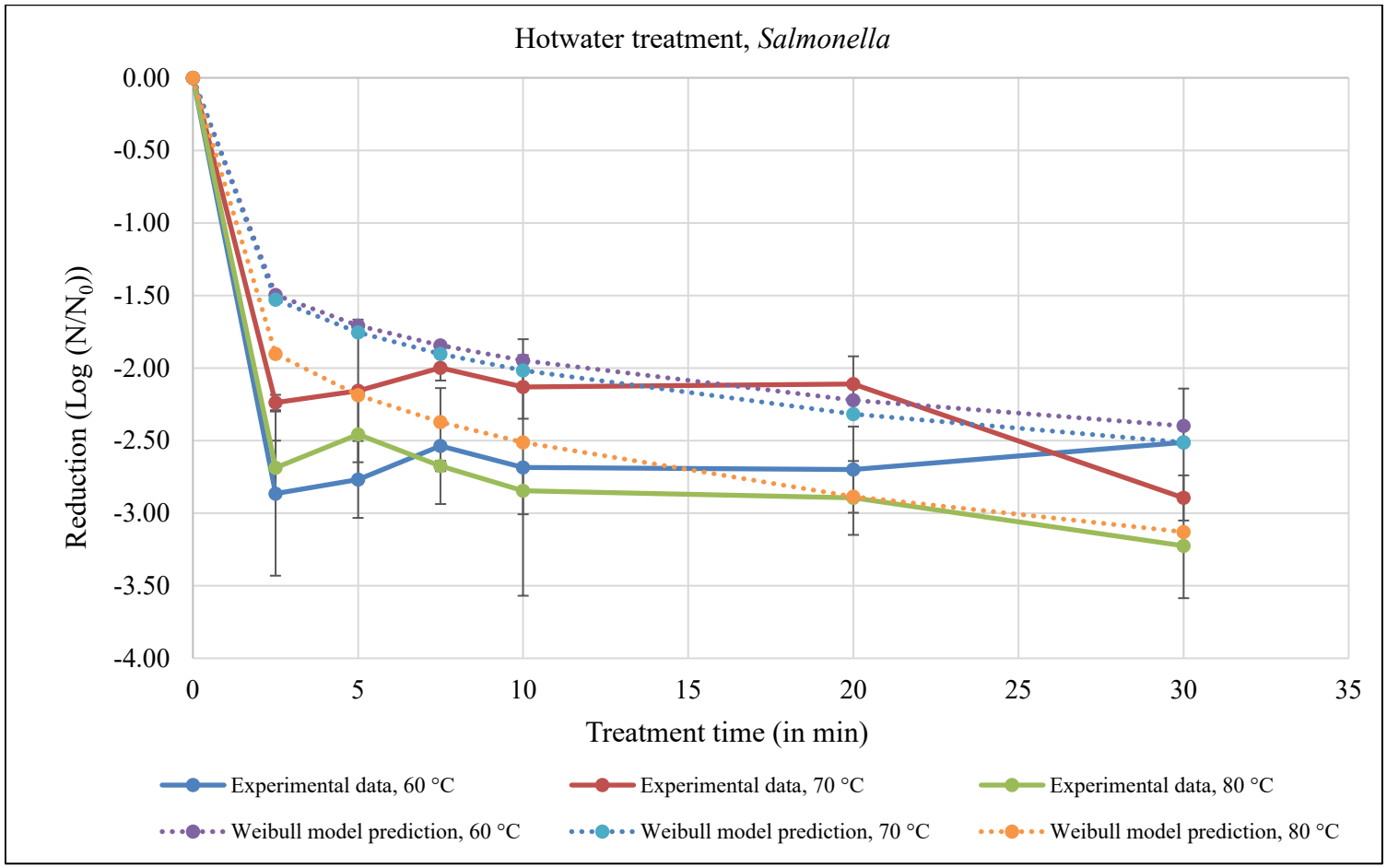


Fig 3.3 Thermal inactivation of *Salmonella* spp., in peanut flour at 60, 70 and 80°C and vertical bars represent standard deviation.

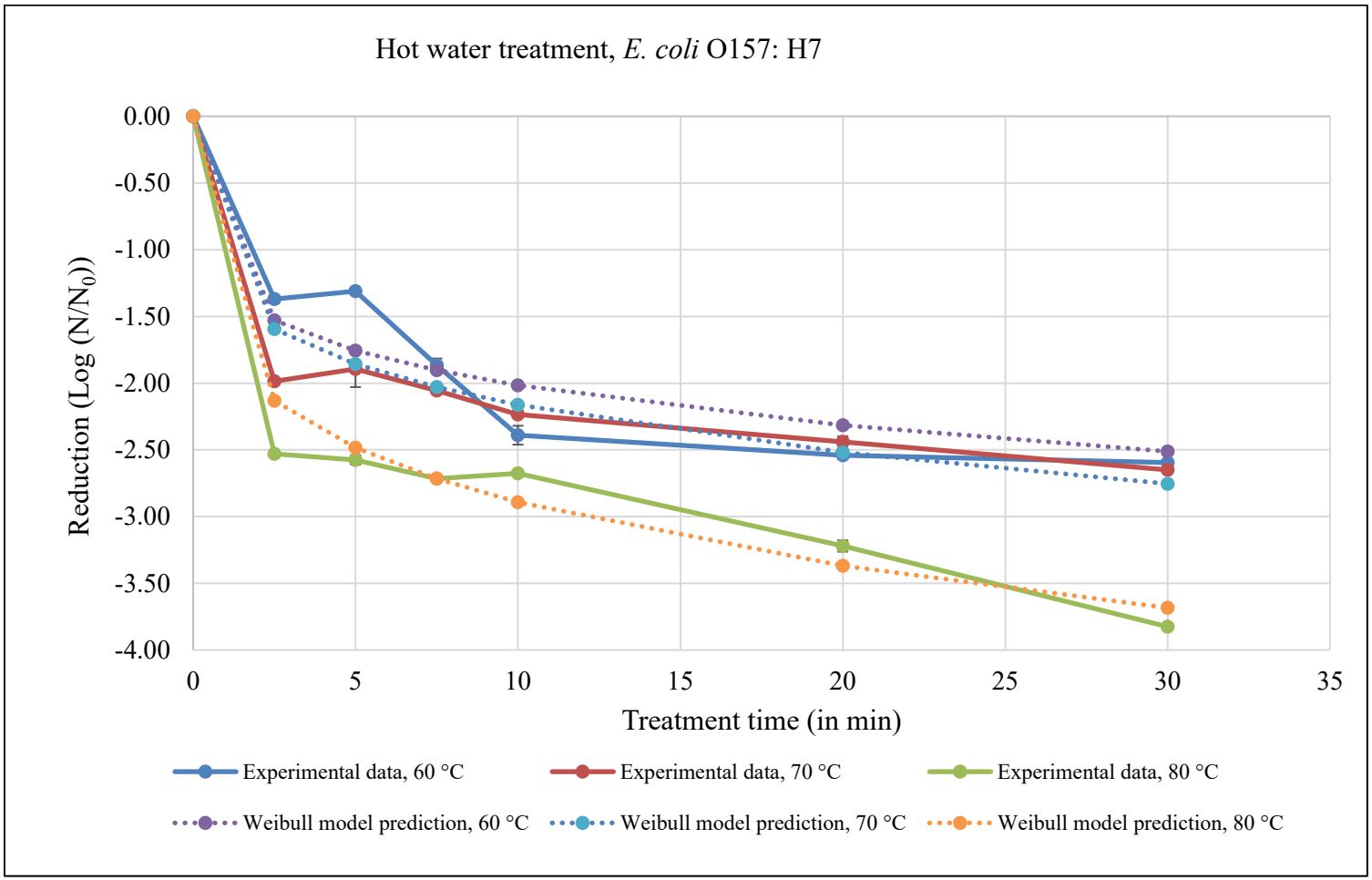


Fig 3.4 Thermal inactivation of *E. coli* O157:H7 in peanut flour at 60, 70 and, 80°C and vertical bars represent standard deviation.

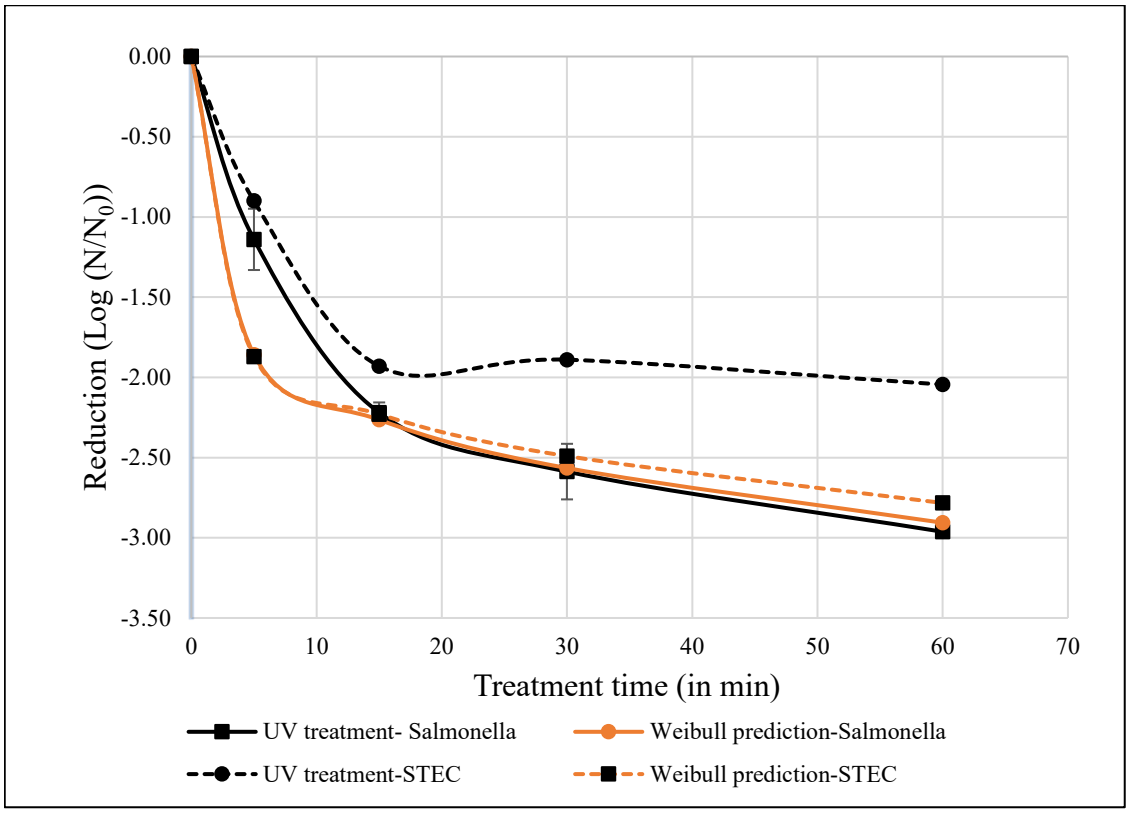


Fig 3.5 UV-C inactivation of *Salmonella* spp., and *E. coli* O157:H7 in peanut flour and vertical bars represent standard deviation.

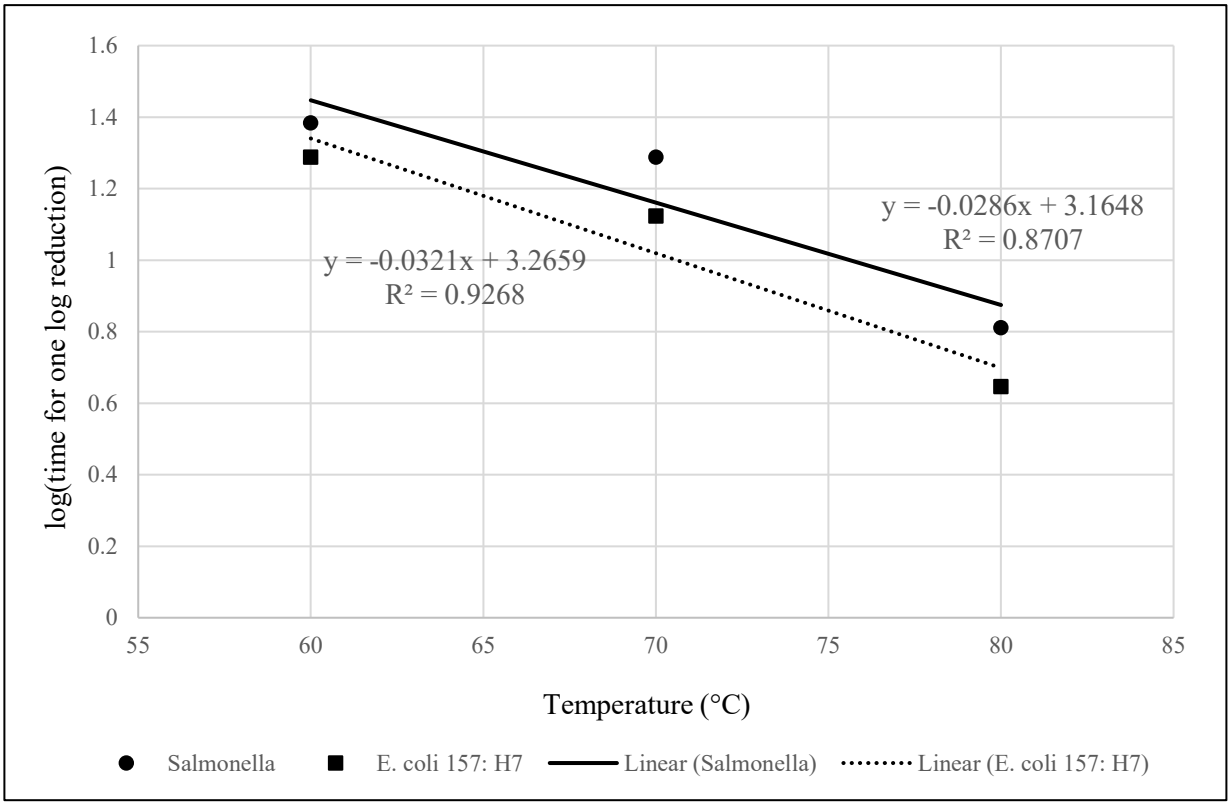


Fig 3.6 Effect of temperature on 1 log CFU/g reduction times of *Salmonella* spp., and *E. coli* O157:H7 in peanut flour is represented using linear regression lines.

Table 3.1 Parameter estimates of log-linear and Weibull models in predicting the inactivation kinetics.

Type of pathogen	Treatment type	Log-linear model		Weibull model parameter estimates					z_T
		D-value (min)	R^2	α	β	SSR	D-value (min)	R^2	(°C)
Salmonella spp.,	60°C	28.49	0.1362	0.3	0.19	4.268	24.18	0.7818	41.92
	70°C	18.95	0.4053	0.3	0.2	0.876	19.42	0.8866	
	80°C	16.56	0.3719	0.1	0.2	0.876	6.47	0.938	
	UV-C	23.74	0.7131	0.16	0.18	0.521	16.46	0.9269	
E. coli O157:H7	60°C	14.5	0.6314	0.3	0.2	0.421	19.42	0.9231	35.09
	70°C	17.81	0.4686	0.3	0.22	0.177	13.29	0.9673	
	80°C	11.89	0.5623	0.08	0.22	0.748	4.43	0.9222	
	UV-C	36.41	0.5609	0.1	0.16	1.936	18.36	0.9023	

D- value refers to the time taken by the process or inactivation technique to decrease bacterial viability by 1 log unit.
Z value refers to the change in temperature required to increase bacterial inactivation by 1 log unit.

CHAPTER IV

CONCLUSIONS AND FUTURE DIRECTIONS

Popular belief and general assumptions suggest that foods with low moisture content or low free water do not support survival of microorganisms (Gurtler, Doyle, & Kornacki, 2014). However, growing evidence shows that foodborne pathogens like *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes* persist in low-moisture conditions and pose the risk of foodborne illnesses (Enache, Podolak, Kataoka, & Harris, 2017). This information is substantiated by increased frequency of outbreaks and recalls related to LMFs in the last decade (Sánchez-Maldonado, Lee, Farber, & technology, 2018). Reports in the literature show that several factors such as type of food, species of organism, extent of contamination and storage conditions, etc., play critical roles in the survival and persistence of pathogens in LMFs (Podolak, Enache, Stone, Black, & Elliott, 2010). In addition, organism's response to environmental stresses and subsequent changes in gene expression leads to the development of resistance to conditions otherwise regarded as detrimental for their growth (Spector, 1998). Based on the literature review conducted for this project three major challenges need immediate scientific attention to address foodborne pathogen contamination in LMFs: 1) Development of methods to recover low bacterial counts from complex food matrices. 2) Understanding the survival kinetics of pathogens in different LMFs under extended and variable storage conditions. 3) Verification and validation of different conventional and novel intervention strategies to

mitigate contamination risk. In this thesis work, we have taken-up challenge # 2 and 3 by focusing our attention on different tree nut and peanut flours utilizing separate approaches. Studies conducted on almond, chestnut and hazelnut flour revealed that the storage RH significantly impacted growth kinetics of *Salmonella* spp. The bacteria displayed improved survival kinetics at low (25% or 45%) when compared to high RH conditions (70%). This finding supports literature reports on persistence of *Salmonella* at low A_w conditions for extended periods. Also, the type of flour seems to have an effect on the bacterial viability. This likely effect can be attributable to differences in chemical composition which needs further research. Although studies on survival kinetics of different types of tree nuts in their raw shelled or unshelled form exist in literature, the findings of this study help to understand risk of acquiring salmonellosis when processed forms of treenuts are prone to post-process contamination and storage at abuse conditions. This information is especially critical in view of changing consumer eating patterns. Since LMFs such as peanut flours are often incorporated into breakfast cereals and protein shakes to boost the protein levels in foods and some consumers have habit of tasting raw cookie dough before baking process (Cheewapramong, Riaz, Rooney, & Lusas, 2002, Wu, Ricke, Schneider, & Ahn, 2017) which is a threat to consumer health. However, one of the main limitations of this study was that initial contamination level is very high (10^{8-9} CFU/g). This is very high level of contamination when we compare with most likely real life contamination levels of maximum 10^{6-7} CFU/g. This is one of the reasons for regulatory agencies such as FDA establishing performance standards for treatment procedures to achieve a 5-log pathogen reduction by juice processors which is also followed by other commodity processors to bring the level of risk to much lower. Hence, future studies would benefit from focusing on much lower initial inoculum load and developing recovery methods with low

detection limits. Furthermore, survival kinetics under dynamic storage conditions to represent farm to fork variations help in better risk analysis, management, and communication.

Experimental results reported in chapter 3 of this thesis evaluated the potential of hot water and UV-C treatment as antimicrobial methods for reducing *Salmonella* and *E. coli* O157: H7 cell counts in peanut flour. Both treatment methods were found to offer great promise for mitigating the risk of these two pathogens. The Weibull model was found better at predicting the inactivation kinetics of evaluated pathogens in peanut flour when compared with linear model. Although infectious dose of *Salmonella* spp., depends upon several factors and 10^6 CFU are presumed to cause disease. Whereas the infectious dose of *E. coli* O157:H7 can be as small as 10-15 cells. Hot water and UV-C treatments studied in this project has for resulted in 3-4 and 2-3 log CFU/g reduction in pathogen counts respectively on peanut flour. This shows that these methods if optimized to achieve > 5 log reductions without affecting the product quality can act as efficient standalone interventions or as a part of multiple hurdle technologies. Both the tested mediums (i.e., Heat and UVC) are widely adopted by many food industries. However, validation and verification of these systems should be carried out on product-by-product basis as not all products behave the same way when exposed to different treatment procedures. Furthermore, understanding the microbial responses at molecular level when subjecting to different treatment methods helps to elucidate differences in log reductions observed between *Salmonella* and STEC in this study. Investigating the reasons for such differences will help understand pathogen phenotypes subjected to different treatment methods. Furthermore, evaluation of gene expression patterns prior, during and post treatment is anticipated to reveal adaptive response mechanisms involved in mediating bacterial survival that could lead to the development and optimization of further mitigation strategies to enhance food safety.

In conclusion, although treatments implemented here do not eliminate bacterial contaminants completely, however they mitigate the risk to some extent. Follow-up studies will need to delve deeper to improve the effectiveness of antimicrobial treatment methods, and most importantly examine the effect of treatments on nutritional quality of tested foods.

REFERENCES

Anderson, N. (2019). Recent advances in low moisture food pasteurization. *Current Opinion in Food Science, 29*, 109-115.

Ballom, K. F., Tsai, H.-C., Taylor, M., Tang, J., & Zhu, M. (2020). Stability of Listeria monocytogenes in non-fat dry milk powder during isothermal treatment and storage. *Food Microbiology, 87*, 103376.

Bansal, A., Jones, T. M., Abd, S. J., Danyluk, M. D., & Harris, L. J. (2010). Most-probable-number determination of Salmonella levels in naturally contaminated raw almonds using two sample preparation methods. *Journal of Food Protection, 73*(11), 1986-1992.

Bedard, B., Kennedy, B., Weimer, A. J. (2014). Geographical information software and shopper card data, aided in the discovery of a Salmonella Enteritidis outbreak associated with Turkish pine nuts. *Epidimeology & Infection*, *142*(12), 2567-2571.

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., . . . Ter Kuile, B. H. (2013a). Low–water activity foods: increased concern as vehicles of foodborne pathogens. *Journal of food protection, 76*(1), 150-172.

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., . . . Ter Kuile, B. H. (2013b). Low–water activity foods: increased concern as vehicles of foodborne pathogens. *Journal of food protection, 76*(1), 150-172.

Beuchat, L. R., Mann, D. A., Kelly, C. A., & Ortega, Y. R. (2017). Retention of viability of Salmonella in sucrose as affected by type of inoculum, water activity, and storage temperature. *Journal of food protection, 80*(9), 1408-1414.

Bevilacqua, A., Speranza, B., Sinigaglia, M., & Corbo, M. R. (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.

Bintsis, T., Litopoulou-Tzanetaki, E., Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry–a critical review. *Journal of Science of Food and Agriculture, 80*(6), 637-645.

Bintsis, T. J. (2017). Foodborne pathogens. *AIMS Microbiology, 3*(3), 529.

Blessington, T., Mitcham, E. J., & Harris, L. J. (2012). Survival of Salmonella enterica,

Escherichia coli O157:H7, and Listeria monocytogenes on inoculated walnut kernels during storage. *Journal of food protection, 75*(2), 245-254.

Blessington, T., Theofel, C. G., Mitcham, E. J., & Harris, L. J. (2013a). Survival of foodborne pathogens on inshell walnuts. *International Journal of Food Microbiology, 166*(3), 341-348.

Brar, P. K., Strawn, L. K., & Danyluk, M. D. (2016). Prevalence, level, and types of Salmonella isolated from North American in-shell pecans over four harvest years. *Journal of Food Protection, 79*(3), 352-360.

Brockmann, S. O., Piechotowski, I., & Kimmig, P. (2004). Salmonella in sesame seed products. *Journal of Food Protection, 67*(1), 178-180.

Burnett, S., Gehm, E., Weissinger, W., & Beuchat, L. (2000). Survival of Salmonella in peanut butter and peanut butter spread. *Journal of Applied Microbiology, 89*(3), 472-477.

Calhoun, S., Post, L., Warren, B., Thompson, S., & Bontempo, A. R. (2013). Prevalence and concentration of Salmonella on raw shelled peanuts in the United States. *Journal of Food Protection, 76*(4), 575-579.

Carroll, C. (2016). Cadbury's salmonella scare: good or bad crisis management? In *The Crisis of Food Brands,* (pp. 45-58): Routledge.

CDC. (1993). Powdered infant formula outbreak. https://www.cdc.gov/mmwr/preview/mmwrhtml/00021081.htm

CDC. (2007a). Multistate Outbreak of Salmonella Wandsworth Infections Linked to Veggie Booty. https://www.cdc.gov/salmonella/2007/veggie-booty-7-18-2007.html

CDC. (2016). STEC outbreak on flour. https://www.cdc.gov/ecoli/2016/o121-06-16/index.html

CDC. (2017). E. coli outbreak on SoyNut butter. https://www.cdc.gov/ecoli/2017/o157h7-03-17/index.html

CDC. (2019). E. coli O26 flour outbreak. https://www.cdc.gov/ecoli/2019/flour-05-19/index.html

Ceylan, E., Fung, D. Y. (2004). Antimicrobial activity of spices 1. *Journal of Rapid Methods and Automation in Microbiology, 12*(1), 1-55.

Cheewapramong, P., Riaz, M., Rooney, L., & Lusas, E. (2002). Use of partially defatted peanut flour in breakfast cereal flakes. *Cereal Chemistry, 79*(4), 586-592.

Chang, A. S., Sreedharan, A., & Schneider, K. R. (2013). Peanut and peanut products: A food safety perspective. *Food Control, 32*(1), 296-303.

Control, C. f. D., & Prevention. (1993). Salmonella serotype Tennessee in powdered milk products and infant formula--Canada and United States, 1993. *42*(26), 516-517.

Control, C. f. D., & Prevention. (2004). Outbreak of Salmonella serotype Enteritidis infections associated with raw almonds--United States and Canada, 2003-2004. *53*(22), 484-487.

Control, C. f. D., & Prevention. (2007). Multistate outbreak of Salmonella serotype Tennessee infections associated with peanut butter--United States, 2006-2007. *56*(21), 521-524.

Control, C. f. D., & Prevention. (2014). Multistate outbreak of Salmonella Braenderup infections linked to nut butter manufactured by nSpired Natural Foods, Inc.(final update). In.

Cordier, J.-L. (2014). Methodological and sampling challenges to testing spices and low-water activity food for the presence of foodborne pathogens. *The microbiological safety of low water activity foods and spices* (pp. 367-386): Springer.

Crowe, S. J., Bottichio, L., Shade, L. N., Whitney, B. M., Corral, N., Melius, B., . . . Allen, K. (2017). Shiga toxin–producing E. coli infections associated with flour. *The New England Journal of Medicine, 377*(21), 2036-2043.

D'Aoust, J.-Y., & Maurer, J. (2007). Salmonella species. In *Food Microbiology: Fundamentals and Frontiers* (pp. 187-236): American Society of Microbiology.

Dai, T., Vrahas, M. S., Murray, C. K., & Hamblin, M. R. (2012). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? *Expert review of anti-infective therapy, 10*(2), 185-195.

Danyluk, M. D., Jones, T. M., Abd, S. J., Schlitt-Dittrich, F., Jacobs, M., & Harris, L. J. (2007). Prevalence and amounts of Salmonella found on raw California almonds. *Journal of Food Protection, 70*(4), 820-827.

Daryaei, H., Sui, Q., Liu, H., Rehkopf, A., Penaloza, W., Rytz, A., . . . Wan, J. (2020). Heat resistance of Shiga toxin-producing Escherichia coli and potential surrogates in wheat flour at two moisture levels. *Food Control, 108*, 106788.

Davidson, G. R., Frelka, J. C., Yang, M., Jones, T. M., & Harris, L. J. (2015). Prevalence of Escherichia coli O157:H7 and Salmonella on inshell California walnuts. *Journal of Food Protection, 78*(8), 1547-1553.

Du, L., Prasad, A. J., Gänzle, M., & Roopesh, M. (2020). Inactivation of Salmonella spp. in wheat flour by 395 nm pulsed light emitting diode (LED) treatment and the related functional and structural changes of gluten. *Food Research International, 127*, 108716.

Eischeid, A. C., Meyer, J. N., Linden, K. G. (2009). UV disinfection of adenoviruses: molecular indications of DNA damage efficiency. *Applied and Environmental Microbiology, 75*(1), 23-28.

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). *Journal of Food Protection, 78*(6), 1106-1112.

Enache, E., Podolak, R., Kataoka, A., & Harris, L. J. (2017). Persistence of Salmonella and other bacterial pathogens in low-moisture foods. In *Control of Salmonella and other bacterial pathogens in low moisture foods* (pp. 67-86): John Wiley & Sons Ltd., Chichester, UK.

Farakos, S. M. S., & Frank, J. F. (2014). Challenges in the control of foodborne pathogens in low-water activity foods and spices. *The microbiological safety of low water activity foods and spices* (pp. 15-34): Springer.

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. *Journal of Food Protection, 80*(5), 879-885.

FDA. (2014). Water acitvity. https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods

Fletcher, S. M., & Shi, Z. (2016). An overview of world peanut markets. *Peanuts*, 267-287.

Food, & Administration, D. (2019). Raw Dough'sa Raw Deal and Could Make You Sick. *FDA. https://www. fda. gov/consumers/consumer-updates/raw-doughs-raw-deal-and-could-make-you-sick. Accessed, 2*.

Forghani, F., den Bakker, M., Liao, J.-Y., Payton, A. S., Futral, A. N., & Diez-Gonzalez, F. (2019a). Salmonella and enterohemorrhagic Escherichia coli serogroups O45, O121, O145 in wheat flour: effects of long-term storage and thermal treatments. *Frontiers in microbiology, 10*, 323.

Frelka, J. C., & Harris, L. J. (2014). Nuts and nut pastes. *The microbiological safety of low water activity foods and spices* (pp. 213-244): Springer.

Funk, J. (2007). Moisture led to Salmonella outbreak. *4*(5), 2007. https://usatoday30.usatoday.com/news/health/2007-04-05-160772288_x.htm

Gabriel, A. A., Tongco, A. M. P., & Barnes Jr, A. A. (2017). Utility of UV-C radiation as anti-Salmonella decontamination treatment for desiccated coconut flakes. *Food Control, 71*, 117-123.

Gautam, B., Govindan, B. N., Gänzle, M., & Roopesh, M. (2020). Influence of water activity on the heat resistance of Salmonella enterica in selected low-moisture foods. *International Journal of Food Microbiology, 334*, 108813.

Gayán, E., García-Gonzalo, D., Álvarez, I., & Condón, S. (2014). Resistance of Staphylococcus aureus to UV-C light and combined UV-heat treatments at mild temperatures. *International Journal of Food Microbiology, 172*, 30-39.

Gomez-Lopez, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. *International Journal of Food Microbiology, 103*(1), 79-89.

Gomez, R. (1977). Nucleic acid damage in thermal inactivation of vegetative microorganisms. In *Advances in Biochemical Engineering, Volume 5* (pp. 49-67): Springer.

Greenwood, M. H., & Hooper, W. (1983). Chocolate bars contaminated with Salmonella napoli: an infectivity study. *The British Medical Journal, 286*(6375), 1394.

Griel, A. E., Eissenstat, B., Juturu, V., Hsieh, G., & Kris-Etherton, P. M. (2004). Improved diet quality with peanut consumption. *Journal of the American College of Nutrition, 23*(6), 660-668.

Gurtler, J. B., Doyle, M. P., & Kornacki, J. L. (2014). The microbiological safety of spices and low-water activity foods: Correcting historic misassumptions. *The microbiological safety of low water activity foods and spices* (pp. 3-13): Springer.

Gurtler, J. B., Keller, S. E., Kornacki, J. L., Annous, B. A., Jin, T., & Fan, X. (2019). Challenges in recovering foodborne pathogens from low-water-activity foods. *Journal of Food Protection, 82*(6), 988-996.

Gyles, C. (2007). Shiga toxin-producing Escherichia coli: an overview. *Journal of Animal Science, 85*(13), 45-62.

Harris, L. J., Lieberman, V., Mashiana, R. P., Atwill, E., Yang, M., Chandler, J. C., . . . Jones, T. (2016). Prevalence and amounts of Salmonella found on raw California inshell pistachios. *Journal of Food Protection, 79*(8), 1304-1315.

He, Y., Guo, D., Yang, J., Tortorello, M. L., Zhang, W. (2011). Survival and heat resistance of Salmonella enterica and Escherichia coli O157:H7 in peanut butter. *Applied and Environmental Microbiology, 77*(23), 8434-8438.

Hijnen, W., Beerendonk, E., & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. *Water Research, 40*(1), 3-22.

Hildebrandt, I. M., Hu, C., Grasso-Kelley, E. M., Ye, P., Anderson, N. M., & Keller, S. E. (2017). Dry transfer inoculation of low-moisture spices containing antimicrobial compounds. *Journal of Food Protection, 80*(2), 338-344.

Hills, B., Manning, C., Ridge, Y., & Brocklehurst, T. (1997). Water availability and the survival of Salmonella typhimurium in porous systems. *International Journal of Food Microbiology, 36*(2-3), 187-198.

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J., Ahmed, R., Middleton, D., . . . Chan, E. (2005). An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of Salmonella Enteritidis. *Journal of Food Protection*, *68*(1), 191-198.

Jeong, S.-G., Baik, O.-D., & Kang, D.-H. (2017). Evaluation of radio-frequency heating in controlling Salmonella enterica in raw shelled almonds. *International Journal of Food Microbiology, 254*, 54-61.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology, 2*(2), 123-140.

Kataoka, A., Enache, E., Black, D. G., Elliott, P. H., Napier, C. D., Podolak, R., & Hayman, M. M. (2014). Survival of Salmonella Tennessee, Salmonella Typhimurium DT104, and Enterococcus faecium in peanut paste formulations at two different levels of water activity and fat. *Journal of food protection, 77*(8), 1252-1259.

Kharel, K., Yemmireddy, V. K., Graham, C. J., Prinyawiwatkul, W., & Adhikari, A. (2018). Hot water treatment as a kill-step to inactivate Escherichia coli O157:H7, Salmonella enterica, Listeria monocytogenes and Enterococcus faecium on in-shell pecans. *LWT-Food Science and Technology, 97*, 555-560.

Kirk, M., Little, C., Lem, M., Fyfe, M., Genobile, D., Tan, A., . . . Lyi, H. (2004). An outbreak due to peanuts in their shell caused by Salmonella enterica serotypes Stanley and Newport–sharing molecular information to solve international outbreaks. *Epidemiology & Infection, 132*(4), 571-577.

Koch, J., Schrauder, A., Alpers, K., Werber, D., Frank, C., Prager, R., . . . Roggentin, P. (2005). Salmonella Agona outbreak from contaminated aniseed, Germany. *Emerging Infectious Diseases, 11*(7), 1124.

Komitopoulou, E., & Peñaloza, W. (2009). Fate of Salmonella in dry confectionery raw materials. *Journal of Applied Microbiology, 106*(6), 1892-1900.

Kornacki, J. L., & Desautels, G. (2014). Dried Dairy-Based Products. *The Microbiological Safety of Low Water Activity Foods and Spices* (pp. 115-126): Springer.

Koseki, S., Nakamura, N., & Shiina, T. (2015). Comparison of desiccation tolerance among Listeria monocytogenes, Escherichia coli O157:H7, Salmonella enterica, and Cronobacter sakazakii in powdered infant formula. *Journal of food protection, 78*(1), 104-110.

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology, 2*(2), 138-155.

Kowalski, W. J., Bahnfleth, W., & Hernandez, M. T. (2009a). A genomic model for predicting the ultraviolet susceptibility of viruses. *Journal of Integrative Neuroscience, 11*(2), 15-28.

Kumar, A., Nayak, R., Purohit, S. R., & Rao, P. S. (2021). Impact of UV-C irradiation on solubility of Osborne protein fractions in wheat flour. *Food Hydrocolloids, 110*, 105845.

Kusumoto, A., Asakura, H., Kawamoto, K. (2012). General stress sigma factor RpoS influences time required to enter the viable but non-culturable state in Salmonella enterica. *Microbiology and Immunology, 56*(4), 228-237

Lahiri, A., Lahiri, A., Iyer, N., Das, P., Chakravortty, D. (2010). Visiting the cell biology of Salmonella infection. *Microbes and Infection, 12*(11), 809-818.

Laroche, C., Fine, F., & Gervais, P. (2005). Water activity affects heat resistance of microorganisms in food powders. *International Journal of Food Microbiology, 97*(3), 307-315.

Lehmacher, A., Bockemühl, J., Aleksic, S. (1995). Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiology and Infection, 115*(3), 501-511.

Little, C., Jemmott, W., Surman-Lee, S., Hucklesby, L., & De Pinna, E. (2009). Assessment of the microbiological safety of edible roasted nut kernels on retail sale in England, with a focus on Salmonella. *Journal of Food Protection, 72*(4), 853-855.

Ly, K. T., & Casanova, J. E. (2007). Mechanisms of Salmonella entry into host cells. Cellular Microbiology, *9*(9), 2103-2111.

Mafart, P., Couvert, O., Gaillard, S., & Leguérinel, I. (2002). On calculating sterility in thermal preservation methods: application of the Weibull frequency distribution model. *International Journal of Food Microbiology, 72*(1-2), 107-113.

Marston, K., Khouryieh, H., Aramouni, F. J. L.-F. S., & Technology. (2016). Effect of heat treatment of sorghum flour on the functional properties of gluten-free bread and cake. *65*, 637-644.

Mattick, K., Jørgensen, F., Legan, J., Lappin-Scott, H., & Humphrey, T. (2000). Habituation of Salmonella spp. at reduced water activity and its effect on heat tolerance. *Applied and Environmental Microbiology, 66*(11), 4921-4925.

McCallum, L., Paine, S., Sexton, K., Dufour, M., Dyet, K., Wilson, M. (2013). An outbreak of Salmonella Typhimurium phage type 42 associated with the consumption of raw flour. *Foodborne Pathogens and Disease, 10*(2), 159-164.

Mead, P. S., & Griffin, P. M. (1998). Escherichia coli O157:H7. *The Lancet, 352*(9135), 1207-1212.

Medus, C., Meyer, S., Smith, K., Jawahir, S., Miller, B., Viger, K., . . . Salehi, E. (2009). Multistate outbreak of Salmonella infections associated with peanut butter and peanut butter-containing products-United States, 2008-2009. *Morbidity and mortality weekly report, 58*(4), 85-90.

Meera, M., Bhashyam, M., Ali, S. (2011). Effect of heat treatment of sorghum grains on storage stability of flour. *LWT-Food Science Technology, 44*(10), 2199-2204.

Miksch, R. R., Leek, J., Myoda, S., Nguyen, T., Tenney, K., Svidenko, V., . . . Samadpour, M. (2013). Prevalence and counts of Salmonella and enterohemorrhagic Escherichia coli in raw, shelled runner peanuts. *Journal of Food Protection, 76*(10), 1668-1675.

Miller, B. D., Rigdon, C. E., Ball, J., Rounds, J. M., Klos, R. F., Brennan, B. M., . . . Smith, K. E. (2012). Use of traceback methods to confirm the source of a multistate Escherichia coli O157:H7 outbreak due to in-shell hazelnuts. *Journal of Food Protection, 75*(2), 320-327.

Mitsuzawa, S., Deguchi, S., & Horikoshi, K. (2006). Cell structure degradation in Escherichia coli and Thermococcus sp. strain Tc-1-95 associated with thermal death resulting from brief heat treatment. *FEMS microbiology letters, 260*(1), 100-105.

Nascimento, M., Carminati, J., Silva, I., Silva, D., Bernardi, A., & Copetti, M. (2018). Salmonella, Escherichia coli and Enterobacteriaceae in the peanut supply chain: From farm to table. *Food Research International, 105*, 930-935.

Nascimento, M. S., Carminati, J. A., Morishita, K. N., Amorim Neto, D. P., Pinheiro, H. P., & Maia, R. P. (2018). Long-term kinetics of Salmonella Typhimurium ATCC 14028 survival on peanuts and peanut confectionery products. *PLOS ONE, 13*(2), e0192457.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic escherichia coli. *Clinical Micorbiology Reviews, 11*(1), 142-201.

Nguyen, H. T., Corry, J. E., Miles, C. A. (2006). Heat resistance and mechanism of heat inactivation in thermophilic campylobacters. *Applied and Environmental Microbiology, 72*(1), 908-913.

Nummer, B., Shrestha, S., & Smith, J. (2012). Survival of Salmonella in a high sugar, low water-activity, peanut butter flavored candy fondant. *Food Control, 27*(1), 184-187.

Nychas, G. (1995). Natural antimicrobials from plants. *New methods of food preservation* (pp. 58-89): Springer.

Ogechukwu, C. O., Ikechukwu, J. O. (2017). Effect of heat processing treatments on the chemical composition and functional properties of lima bean (Phaseolus lunatus) flour. *American Journal of Food Sciences and Nutrition, 1*(1), 14-24.

Park, E. J., Oh, S. W., & Kang, D. H. (2008). Fate of Salmonella Tennessee in peanut butter at 4 and 22 C. *Journal of Food Science, 73*(2), 82-86.

Park, M.-J., Kim, J.-H., & Oh, S.-W. (2019). Inactivation effect of UV-C and mild heat treatment against Salmonella Typhimurium and Escherichia coli O157:H7 on black pepper powder. *Food science and biotechnology, 28*(2), 599-607.

Paton, J. C., & Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. *Clinical Microbiology Reviews, 11*(3), 450-479.

Peleg, M. (2006). Isothermal microbial heat inactivation. *Advanced Quantitative Microbiology for Foods and Biosystems,* 1-48.

Pérez, I. C., Mu, T.-H., Zhang, M., Ji, L.-L. (2017). Effect of heat treatment to sweet potato flour on dough properties and characteristics of sweet potato-wheat bread. *Food Science and Technology International, 23*(8), 708-715.

Pinkas, J. M., Battista, K., & Morille-Hinds, T. (2009). Microbiological spoilage of spices, nuts, cocoa, and coffee. *Compendium of the Microbiological Spoilage of Foods and Beverages* (pp. 325-350): Springer.

Pinkas, J. M., & Keller, S. E. (2014). Spices. *The microbiological safety of low water activity foods and spices* (pp. 99-114): Springer.

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. *Journal of Food Protection, 73*(10), 1919-1936.

Popoff, M. Y., Le Minor, L. E. (2015). Salmonella. *Bergey's Manual of Systematics of Archea and Bacteria,* 1-1.

Quinn, A. R., Liao, R. F., Steele, F. M., Jefferies, L. K., & Taylor, B. J. J. F. C. (2021). Isothermal inactivation of Salmonella, Listeria monocytogenes, and Enterococcus faecium NRRL B-2354 in peanut butter, powder infant formula, and wheat flour. *121*, 107582.

Rifna, E., Singh, S. K., Chakraborty, S., & Dwivedi, M. (2019). Effect of thermal and non-thermal techniques for microbial safety in food powder: Recent advances. *Food Research International, 126*, 108654.

Roberts, T., Cordier, J.-L., Gram, L., Tompkin, R., Pitt, J., Gorris, L., & Swanson, K. (2005). Nuts, oilseeds, and dried legumes. *Micro-Organisms in Foods 6* (pp. 440-466): Springer.

Rose, D., Bianchini, A., Martinez, B., & Flores, R. (2012). Methods for reducing microbial contamination of wheat flour and effects on functionality. *Cereal Foods World, 57*(3), 104.

Rowe, B., Hutchinson, D., Gilbert, R., Hales, B., Begg, N., Dawkins, H., . . . Jepson, M. (1987). Salmonella ealing infections associated with consumption of infant dried milk. *The Lancet, 330*(8564), 900-903.

Rushdy, A., Stuart, J., Ward, L., Bruce, J., Threlfall, E., Punia, P., Bailey, J.R. (1998). National outbreak of Salmonella senftenberg associated with infant food. *Epidemiology & Infection, 120*(2), 125-128.

Salazar, J. K., Natarajan, V., Stewart, D., Suehr, Q., Mhetras, T., Gonsalves, L. J., & Tortorello, M. L. (2019a). Survival kinetics of Listeria monocytogenes on chickpeas, sesame seeds, pine nuts, and black pepper as affected by relative humidity storage conditions. *PloS one, 14*(12), e0226362.

Sánchez-Maldonado, A. F., Lee, A., Farber, J. M. (2018). Methods for the control of foodborne pathogens in low-moisture foods. *Annual Review of Food Science and Technology, 9*, 177-208.

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journla of Food Protection, 75*(1), 123-131.

Shachar, D., & Yaron, S. (2006). Heat tolerance of Salmonella enterica serovars Agona, Enteritidis, and Typhimurium in peanut butter. *Journal of Food Protection, 69*(11), 2687-2691.

Smelt, J., Brul, S. (2014). Thermal inactivation of microorganisms. *Critical Reviews in food science and nutrition, 54*(10), 1371-1385.

Spector, M. P. (1998). The starvation-stress response (SSR) of Salmonella. *Advances in microbial physiology, 40*, 233-279.

Strawn, L. K. (2009). Fate of Escherichia Coli O157:H7 and Salmonella Spp. on Fresh and Frozen Cut Mangoes, Papayas and Pineapples. University of Florida

Sun, Q., Gong, M., Li, Y., & Xiong, L. (2014). Effect of dry heat treatment on the physicochemical properties and structure of proso millet flour and starch. *Carbohydrate Polymers, 110*, 128-134.

Syamaladevi, R. M., Adhikari, A., Lupien, S. L., Dugan, F., Bhunia, K., Dhingra, A., & Sablani, S. S. (2015). Ultraviolet-C light inactivation of Penicillium expansum on fruit surfaces. *Food Control, 50*, 297-303.

Tarr, P. I., Gordon, C. A., & Chandler, W. L. (2005). Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *365*(9464), *The Lancet,* 1073-1086.

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 Microbiology, 82*, 135-141.

van Boekel, M. A. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *International Journal of Food Microbiology, 74*(1-2), 139-159.

Vasser, M., Barkley, J., Miller, A., Gee, E., Purcell, K., Schroeder, M. N. (2021). Notes from the Field: Multistate Outbreak of Escherichia coli O26 Infections Linked to Raw Flour—United States, 2019. *Morbidity and Mortality Weekly Reports, 70*(16), 600.

Wu, S., Ricke, S. C., Schneider, K. R., & Ahn, S. (2017). Food safety hazards associated with ready-to-bake cookie dough and its ingredients. *Food Control, 73*, 986-993.

Xu, J., Song, J., Tan, J., Villa-Rojas, R., Tang, J. (2020). Dry-inoculation methods for low-moisture foods. *Trends in Food Science and Technology, 103, 68-77*

Yada, S., & Harris, L. (2018). Recalls of tree nuts and peanuts in the US, 2001 to present (version 2)[Table and references]. *US recalls of nuts. Available at: http://ucfoodsafety. ucdavis. edu/files/162415. pdf. Accessed, 5*.

BIOGRAPHICAL SKETCH

Rajat Sharma completed high school from Dr. S. K. Memorial Public School, Moga, Punjab, India in 2012. He enrolled at Punjab Agricultural University, Ludhiana, India where he completed his Bachelor's in Agricultural Sciences (Hons.) in summer 2018. Fall 2018 he traveled to the United States to pursue graduate studies with the intent to establish a research career. During summer 2020, he joined Dr. Yemmireddy's lab where he fulfilled the requisites for a M.S and earned his degree in July of 2021. Rajat Sharma's contact email: rajatsharma1042@gmail.com.