

EFFICACY OF CONSUMER-AVAILABLE ANTIMICROBIALS FOR THE DISINFECTION
OF PATHOGEN CONTAMINATED GREEN BELL PEPPER AND EFFICACY OF
CONSUMER CLEANING METHODS FOR THE DECONTAMINATION OF KNIVES

A Thesis

by

KEILA LIZTH PEREZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2010

Major Subject: Food Science and Technology

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ABSTRACT

Efficacy of Consumer-Available Antimicrobials for the Disinfection of Pathogen Contaminated Green Bell Pepper and Efficacy of Consumer Cleaning Methods for the Decontamination of Knives. (May 2010)

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Dr. Alejandro Castillo

Limited information exists regarding the efficacy of consumer-available antimicrobials for the use on produce surfaces. There is a strong focus on eliminating pathogens from produce at a commercial level, but consumers can achieve pathogen reduction in a domestic setting. The objectives were to determine the ability of consumer-available antimicrobials to disinfect waxed green bell peppers, determine the efficacy of knife cleaning methods, and assess the transfer of contamination.

Peppers were inoculated via immersion in a cocktail of rifampicin-resistant *Salmonella* serovars and *Escherichia coli* O157:H7 to a final concentration of 5.6 ± 0.5 \log_{10} CFU/cm². In Study 1, samples of 3 10-cm² pieces of inoculated pepper were excised from smooth tissue and immersed in 3% (v/v) hydrogen peroxide (H₂O₂), 2.5% (v/v) acetic acid, 70% (v/v) ethyl alcohol (EtOH), or sterile distilled water (SDW) for various lengths of time. Following treatment, samples were immersed for 30 s in a neutralizer solution. For Study 2, inoculated peppers were chopped into 1-cm² pieces.

Knives were treated with one cleaning method: no treatment (control), towel wipe (TW), running hot water for 5 s (5SW), running hot water for 10 s (10SW) or 1% (v/v) detergent solution followed by hot running water for 10 s (ST). After treatments, knives were used to chop cucumbers. Surviving *Salmonella* and *E. coli* O157:H7 for both studies were selectively enumerated on lactose-sulfite-phenol red-rifampicin agar following aerobic incubation of plates for 24 h at 35 °C.

Hydrogen peroxide exposure for 5 min resulted in reductions of $1.3 \pm 0.3 \log_{10}$ CFU/cm² for both pathogens. Following 1 min exposure to EtOH, pathogens were reduced by $1.3 \pm 0.1 \log_{10}$ CFU/cm²; exposure for >1 min did not result in additional reduction. Acetic acid exposure after 5 min resulted in a *Salmonella* reduction of $1.0 \pm 0.7 \log_{10}$ CFU/cm², but for *E. coli* O157:H7, exposure resulted in no significant reduction ($p < 0.05$) of pathogens compared to SDW at the various points. For Study 2, 5SW, 10SW, and ST were equally effective for knife decontamination. No significant difference ($p < 0.05$) was found between \log_{10} CFU/cm² on knife blade and \log_{10} CFU/cm² transferred to surface of cucumber; therefore, viable organisms remaining on the knife blade were transferred onto the surface of the cucumber.

Findings suggest EtOH and H₂O₂ may be effective consumer-deployable antimicrobials for surface decontamination of smooth produce, and contaminated produce can contaminate other produce. Further research of antimicrobial exposure on produce sensorial characteristics is also advised in order to determine how various antimicrobial exposure times will affect the quality and sensorial characteristics of the produce commodity.

DEDICATION

To my family and friends,
thank you for the patience, understanding, encouragement, and love you have bestowed
on me throughout these past years,
and to the memory of my grandmother, Isidora Ramos, who always believed in me and
motivated me to excel.

ACKNOWLEDGMENTS

I would like to thank my committee Dr. Matthew Taylor, Dr. Alejandro Castillo and Dr. Luis Cisneros-Zevallos for their guidance and support throughout the course of this research.

Thank you also to my colleagues, lab mates, student workers, and the department faculty and staff especially Ms. Veronica Rosas and Mrs. Lisa Lucia for all your support. I appreciate and value your assistance both inside and outside the laboratory. I am blessed to have had the opportunity to work with each one of you, and you all have made my time at Texas A&M University a great experience that I will always cherish.

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TABLE OF CONTENTS

	Page
ABSTRACT	iii
DEDICATION	v
ACKNOWLEDGMENTS.....	vi
TABLE OF CONTENTS	vii
LIST OF FIGURES.....	ix
LIST OF TABLES	x
CHAPTER	
I PRODUCE CONTAMINATION	1
Produce-borne Illness	1
Sources of Produce Contamination	2
Bacterial Growth on Produce	5
Bell Pepper Physiology, Harvesting, and U.S. Consumption	7
II PRODUCE-BORNE <i>ESCHERICHIA COLI</i> O157:H7 AND <i>SALMONELLA ENTERICA</i>	10
<i>Escherichia coli</i> O157:H7 Growth Requirements	10
Virulence Groups of <i>E. coli</i>	11
Epidemiological and Economic Impact of <i>E. coli</i> O157:H7	12
<i>E. coli</i> O157:H7 Outbreaks in Fresh Produce	12
<i>Salmonella</i> Classification and Growth Requirements.....	14
Salmonellosis	15
Epidemiological and Economic Impact of <i>Salmonella</i>	16
<i>Salmonella</i> Outbreaks in Fresh Produce	17
III CONSUMER IN-HOME PRACTICES AND CLEANING METHODS.....	19
Consumer Practices	19

CHAPTER	Page
Vinegar as a Consumer Available Antimicrobial	21
Hydrogen Peroxide as a Consumer Available Antimicrobial	25
Ethanol as a Consumer Available Antimicrobial	26
Consumer Methods of Knife Handling	27
 IV MATERIALS AND METHODS	 31
Bacterial Culture Preparation and Maintenance.....	31
Preliminary Experiments	31
Dip Inoculation Validation.....	31
Spot Inoculation Validation	33
Bacterial Lawn Method Validation	34
Swab and Sponge Validation	34
Chopping Validation	35
Study 1: Antimicrobial Disinfection of Pepper Surfaces	36
Study 2A: Efficacy of Decontamination of Knives	38
Study 2B: Transfer Testing	39
Statistical Analysis	40
 V RESULTS	 41
Preliminary Studies	41
Dip Inoculation Validation.....	41
Spot Inoculation Validation	42
Bacterial Lawn Method Validation	44
Sponge and Swab Validation	44
Chopping Validation	45
Study 1: Antimicrobial Disinfection of Pepper Surfaces	46
Study 2A: Efficacy of Decontamination of Knives	48
Study 2B: Transfer Testing	50
 VI DISCUSSION	 54
 VII CONCLUSIONS	 59
 REFERENCES.....	 61
 VITA	 69

LIST OF FIGURES

FIGURE		Page
1	Mechanisms of fresh produce contamination.....	4
2	Conditions for bacterial growth.....	6
3	Bell pepper availability and U.S. population 1980-2008	9
4	Vinegar manufacture flow diagram.....	22
5	Efficacy of decontamination of knife through various cleaning methods for <i>Salmonella</i> serovars	49
6	Efficacy of decontamination of knife through various cleaning methods for <i>E. coli</i> O157:H7	50
7	Transfer of <i>Salmonella</i> serovars on the surfaces of contaminated green bell peppers to non-treated salad cucumbers via contaminated knives after various cleaning methods	52
8	Transfer of <i>E. coli</i> O157:H7 on the surfaces of contaminated green bell peppers to non-treated salad cucumbers via contaminated knives after various cleaning methods	53

LIST OF TABLES

TABLE		Page
1	Microbial pathogens associated with produce-borne disease outbreaks	3
2	Bacterial attachment to bell pepper by dipping at various time points	41
3	Spot inoculation validation results	43
4	Sponge and swab validation results	44
5	Chopping validation results.....	46
6	Reduction of <i>Salmonella</i> serovars on bell peppers after various times of contact with different antimicrobials.....	47
7	Reduction of <i>E. coli</i> O157:H7 on bell peppers after various times of contact with different antimicrobials.....	48

CHAPTER I

PRODUCE CONTAMINATION

Produce-borne Illness

An estimated 76 million people in the United States each year become ill from pathogens in food with individuals such as children, elderly, pregnant women, and immune-compromised individuals being more susceptible to foodborne illnesses than others (Mead and others 1999). According to the U.S. Centers for Disease Control and Prevention (CDC), there are 325,000 hospitalizations and 5,000 deaths related to foodborne diseases each year (CDC 2005). The number of foodborne diseases has increased over the last years because of different factors including consumption, change in consumers' habits, and complex distribution systems (FDA 2009a; FSIS 2006). The increased use of salad bars and the number of meals being eaten outside the home has increased the risk of food handling errors with fresh produce, thus increasing the number of persons exposed to produce-contaminating pathogens (de Roever 1998). Per capita consumption of fresh fruits and vegetables has increased from 254 lbs in 1980 to 328 lbs in 2000 (Matthews 2006). Between 1980 and 2001, fresh vegetable imports increased by over 250%, while fresh fruit imports increased by 155% (James 2006). Consumption is expected to continue to increase due to fresh fruits and vegetables being a key component in programs designed to address healthier eating habits (Matthews 2006). This increase in per capita consumption along with the increase in importation of produce from regions where standards of growing and handling produce may be

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compromised has resulted in an increased interest in outbreaks attributed to contamination of fresh produce, especially salad vegetables (Beuchat 1995). As consumption of fresh produce increases, the incidence of foodborne illness also increases; the median number of reported produce-associated outbreaks increased from 2 outbreaks per year in the 1970s, to 16 per year in the 1990s (Sivapalasingam and others 2004).

Sources of Produce Contamination

Produce can become contaminated with pathogens during production, harvest, and processing, and at retail outlets, in foodservice establishments, and in the home kitchen via improper handling and storage prior to consumption (Kader 2002; Johnston and others 2005). Even transportation by consumers can affect the microbial safety of the produce (Brackett 1999). The major source of microbial contamination of fresh produce is associated with human or animal feces (Kader 2002). From 1973 to 1997, a total of 190 produce-associated outbreaks were reported, resulting in 16,058 illnesses, 598 hospitalizations, and 8 deaths; produce-associated outbreaks rose from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam and others 2004). From 1990 to 2002, 187 produce-associated outbreaks were linked to specific etiological agents; 102 (55%) were caused by bacteria, 68 (36%) were caused by viruses, and 17 (9%) were caused by parasites (James 2006). Microbial pathogens have been found to be associated with produce-borne disease outbreaks as depicted by Table 1.

Table 1- Microbial pathogens associated with produce-borne disease outbreaks.

Organism	Produce Item Associated with Outbreak	Confirmed Cases	Country affected by Outbreak	Year	Reference
<i>Salmonella</i> (<i>S. Saintpaul</i>)	Jalapeño and serrano peppers	1,440	United States	2008	(CDC 2008)
<i>Escherichia coli</i> O157:H7	Spinach	199	United States	2006	(Maki 2006)
<i>Campylobacter</i>	Tomato and cucumber salad	27	Australia	2001	(Unicomb and others 2009)
<i>Cyclospora</i>	Imported raspberries	1,465	United States and Canada	1996	(Herwaldt and others 1997)
<i>Yersinia pseudotuberculosis</i>	Carrots	400	Finland	2006	(Rimhanen-Finne and others 2008)
<i>Shigella sonnei</i>	Imported baby corn	218	Denmark and Australia	2007	(Lewis and others 2007)

According to the CDC (2005), a number of foodborne disease outbreaks have been traced to fresh fruits and vegetables processed under less than sanitary conditions such as alfalfa sprouts because the conditions under which they are sprouted are ideal for growing microbes and because they are eaten without further cooking. These outbreaks show that the quality of the water used for washing after harvest is critical (CDC 2005), and when water comes in contact with produce, the quality of the water dictates the potential for contamination (Kader 2002). Water used to apply pesticides to plants and

for post-harvest cooling and processing can transfer microbes directly to the produce, unless the water is treated to potable standards, and water used for irrigation may also be a source of contamination if it is contaminated surface water and if during irrigation it comes in contact with the edible portions of the plant (Lynch and others 2009). In addition, many crops will receive supplemental irrigation and protective topical sprays mixed with the water. Many commodities are cooled, moved/conveyed, or washed with water prior to their sale (Matthews 2006). Thus, water quality plays an important role in pre- and post-harvest microbiological quality of fruits and vegetables. Pathogenic bacteria such as *Salmonella* have been isolated from irrigation water and have been transmitted by direct contact to the water to other areas of production, including the workers (Gallegos-Robles and others 2008).

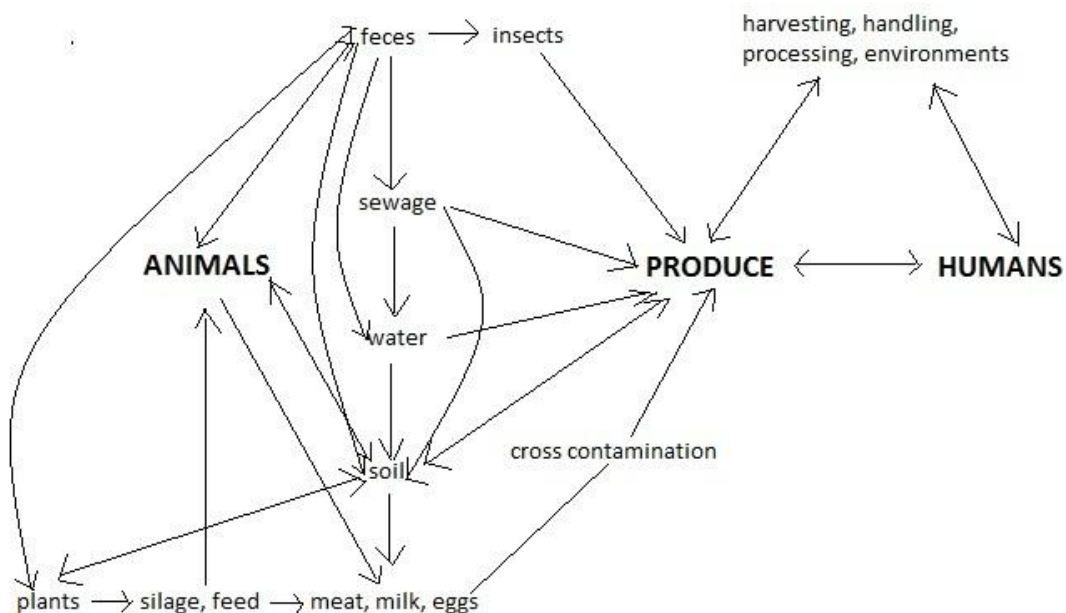


Figure 1- Mechanisms of fresh produce contamination. Mechanisms by which fresh produce might become contaminated with pathogens and/or serve as vehicles for human disease (Beuchat 1995).

As shown in Figure 1, there are many mechanisms/routes by which fresh produce can become contaminated with pathogenic microorganisms. Other pre-harvest sources of pathogenic microorganisms on fresh produce include soil, green or inadequately composted manure, air (dust), and wild and domestic animals (Beuchat 1995). Alvarado-Casillas and others (2007) indicated that select produce commodities such as fresh cantaloupes and bell peppers could be sanitized at the packing facility, but the method should not be used to replace overall good hygiene practices. Worker hygiene and sanitation practices during production, harvesting, sorting, packing, and transport play a critical role in minimizing the potential for microbial contamination of fresh produce, and microorganisms mostly originate from enteric environments, intestinal tract and fecal material of humans or animals, and the survival and/or growth of the pathogen on the produce item is influenced by the organism, produce item, and conditions of storage (Kader 2002).

Bacterial Growth on Produce

Certain conditions can inhibit the growth of bacteria on produce while other conditions will actually facilitate and favor the growth of bacteria. Microorganisms residing on fresh and fresh-cut produce, throughout the journey from farm to fork, will undergo cycles of subjection to unfavorable and hostile environments, periods of limited growth, along with periods of growth when conditions are favorable (James 2006). A study by Jiménez and others (2007) isolated *Salmonella* serotypes from pepper production systems; they suggested that green bell peppers represents a possible carrier of human pathogens and may be involved in sporadic and transient disease. Liao and

Cooke (2001) showed that surfaces of injured fruit tissue are the principal sites for bacterial attachment, and a small portion of the bacteria attached to the tissue are resistant to the sanitizer treatment. Further, these researchers suggested that avoiding mechanical injuries to fresh fruits during and after harvest would reduce the chance of pathogen attachment and contamination on green pepper and fruits of similar nature (de Roever 1998). Thus, transmission of microorganisms from their potential reservoirs to each fruit and vegetable is different since each fruit and vegetable has a unique combination of composition and physical characteristics, growing and harvesting practices, cooling techniques, and optimum storage temperatures and environment as seen in Figure 2 (de Roever 1998).

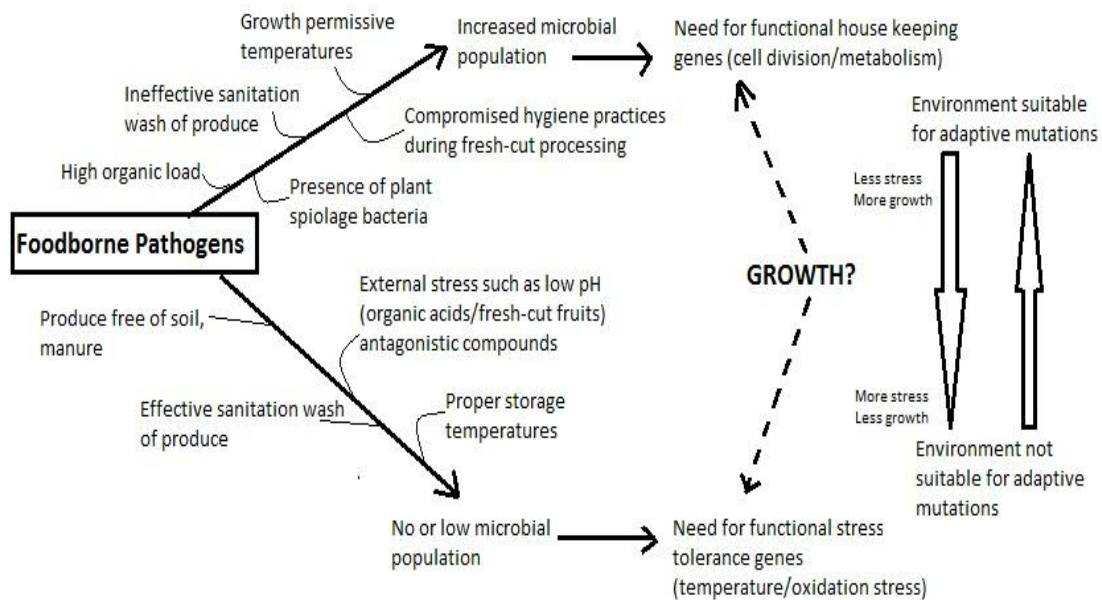


Figure 2- Conditions for bacterial growth. Certain conditions will inhibit bacterial growth while other more favorable conditions will facilitate the growth of bacteria (James 2006).

Bell Pepper Physiology, Harvesting, and U.S. Consumption

Peppers belong to the taxonomic family *Solanaceae*, as do potatoes and tomatoes (Kozukue and others 2005). A variety of green bell peppers (*Capsicum annuum* L.) are grown for both fresh market and minimal processing (Hartz and Cantwell 2008) with 16% being utilized in processed products (canned, frozen, dried) (Lucier and Lin 2001). Bell peppers are non-climacteric, do not ripen after harvest, and ethylene does not enhance ripening of partially colored peppers (Gross and others 2004; Kader 2002). Bell peppers may be harvested at the immature (green) stage or after the mature color (i.e. red, orange, or yellow) depending on the variety (Hartz and Cantwell 2008). Criteria for maturity include size, firmness, and color, and sizes include small, medium, large, and extra large/jumbo (Gross and others 2004). Nearly all bell peppers are harvested by hand into bulk bins or trailers for transportation to packing facilities and a typical field of fresh-market peppers is harvested by hand every week or so over the course of about a month (Hartz and Cantwell 2008; Lucier and Lin 2001). Peppers can be graded into U.S. Fancy, U.S. No. 1 and U.S. No. 2 based primarily on external appearance, but those that are not graded are “unclassified” (Gross and others 2004). Bell peppers are grown in 48 states with the largest concentrations in Florida and California and marketed year-round; domestic shipments peak during May and June and import shipments during winter months (Lucier and Lin 2001). The peppers should be stored at a temperature range of 7-10 °C (Kader 2002); peppers are sensitive to chilling injury which can cause symptoms such as surface pitting, water-soaking, decay and discoloration of seed cavity (Gross and others 2004). Delays to cooling should be less than 9 h at 20-25 °C and less than 6 h at

37 °C in order to not reduce visual quality, glossiness, and firmness (Cantwell and Thangaiyah 2001). The approximate storage life of bell peppers is 2-3 wks under optimum temperature and storage conditions (Kader 2002). A food-grade edible wax which may contain carnauba wax, shellac wax, paraffin wax, candelilla wax, and/or bee wax is applied to the majority of commercially produced peppers to reduce moisture loss and scuffing during marketing which can extend the storage life, under ideal conditions up to 3 wks (Lucier and Lin 2001; Thirupathi and others 2006).

According to data from the U.S. Department of Agriculture (USDA) Agricultural Research Service's 1994-96 Continuing Survey of Food Intakes by Individuals, on any given day, 24% of Americans will consume bell peppers with their meal which can be compared to the 28% of fresh-market tomatoes (USDA 1998). Furthermore, 63% of the bell peppers consumed are consumed at home (Lucier and Lin 2001). In 2008, per capita consumption of bell peppers increased to 9.9 lbs farm weight compared to 2.9 lbs in 1998 (USDA 2009b). Also, as seen in Figure 3, as the population of the U.S. has grown, so has the retail per capita availability of bell peppers. Different reasons for this rise in consumer use of bell peppers could include the wider range of food that include bell peppers as an ingredient, wider availability of high-quality hot-house and colored peppers, increased away from home dining, consumer recognition of the nutritional quality of vegetables, and the increased diversity of the nation's population (Lucier and Lin 2001). Most peppers are eaten raw in salads and salsa, processed by canning, freezing, and pickling (Gross and others 2004). Therefore, bell peppers can serve as an

important component of meals prepared in the home while also providing a potential reservoir for microorganisms if the conditions are favorable.

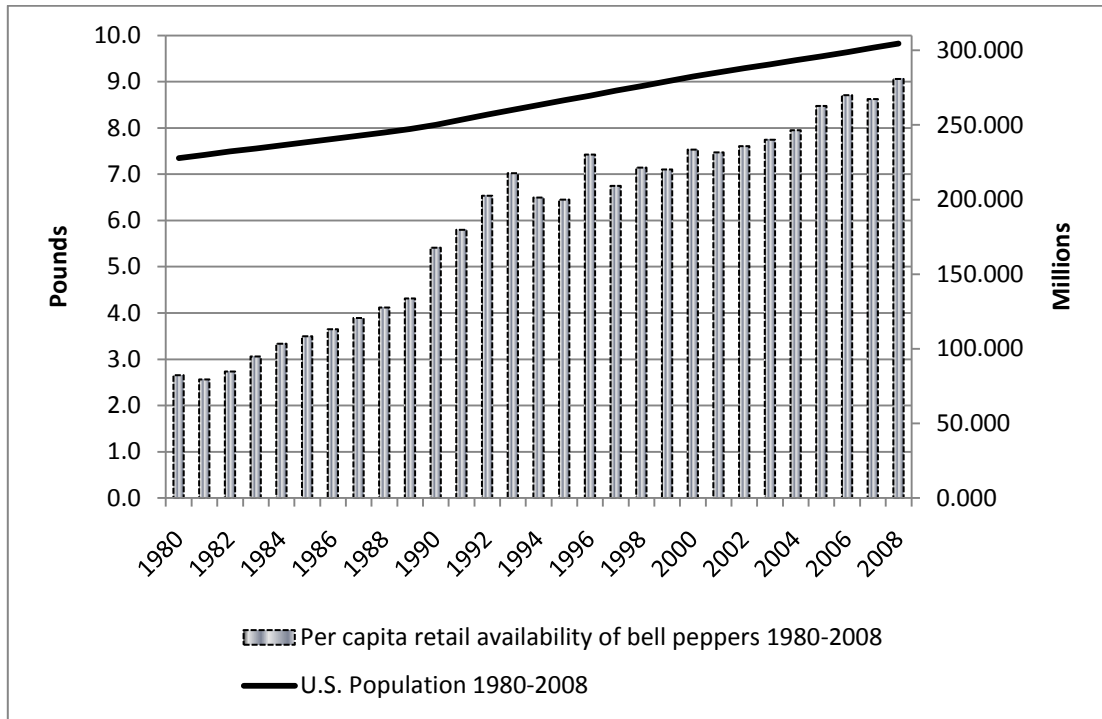


Figure 3- Bell pepper availability and U.S. population 1980-2008. The U.S. population continues to grow over time along with the per capita retail availability of bell peppers (USDA 2010).

CHAPTER II

PRODUCE-BORNE *ESCHERICHIA COLI* O157:H7 AND *SALMONELLA ENTERICA*

***Escherichia coli* O157:H7 Growth Requirements**

Escherichia coli belongs to the family *Enterobacteriaceae*; within the genus, there are six species (*E. hermannii*, *E. fergusonii*, *E. vulneris*, *E. blattae*, *E. albertii*, and *E. coli*) (Willshaw and others 2000). The *E. coli* are Gram-negative facultatively anaerobic bacilli. Strains that possess flagella are motile with peritrichous flagella (Darnton and others 2007). Most strains ferment glucose with production of acid and gas, and lactose is fermented with production of both acid and gas by most strains (Willshaw and others 2000). The organism is catalase positive, cytochrome oxidase negative, indole positive, Voges-Proskauer negative, and methyl red positive. Strains are unable to utilize citrate as their sole carbon source whereas they are able to use acetate as a sole carbon source (Willshaw and others 2000). *E. coli* O157:H7 differs from other strains of *E. coli* by being slow or unable to ferment sorbitol and by the lack of the lysosomal enzyme β -glucuronidase (Tortorello 1999). *E. coli* are mesophilic and can grow within a temperature range of 15-45 °C, and the optimum temperature for growth is 37 °C (Willshaw and others 2000). The optimum reported minimum pH value for growth of *E. coli* O157:H7 is 4.5 (Jay and others 2005). *E. coli* O157:H7 cannot grow at the high end of the temperature range (44-45 °C) but can tolerate acid conditions as low as pH 2.5 (Tortorello 1999). Under optimum conditions the minimum water activity for the growth of *E. coli* has been reported to be 0.96 (Jay and others 2005).

Virulence Groups of *E. coli*

There are five recognized virulence groups for *E. coli*: enteroaggregative (EAEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), and enterotoxigenic (ETEC). *E. coli* O157:H7 belongs to the EHEC virulence group which produce large quantities of Shiga-like toxins (Jay and others 2005) that are closely related to the toxins produced by *Shigella dysenteriae* (FDA 2006a). EHEC strains possess the chromosomal gene *eaeA* and produce attachment-effacement (A/E) lesions; these strains affect only the large intestine (Jay and others 2005) and cause severe damage to the lining of the intestine (FDA 2006a). The pathogenicity of EHEC is due to the possession of Stx toxins, endotoxins, and host-derived cytokines; Stx1 and Stx2 toxins inhibit protein synthesis in endothelial cells and are important risk factors of disease severity (Jay and others 2005; Dean-Nystrom and others 1998; Kawano and others 2008). The A/E lesion begins as a non-intimate attachment of the bacterium, followed by the injection of type III proteins, which effect cytoskeletal changes and effacement of microvilli (Jay and others 2005). The EHEC virulence group causes symptoms such as profuse, bloody diarrhea (hemorrhagic colitis), abdominal pain, and fever is usually absent or low-grade (Tortorello 1999; FDA 2006a). Complications of this disease can lead to Hemolytic Uremic Syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and kidney failure which may cause death (Tortorello 1999), but with intensive care, the death rate for hemolytic uremic syndrome is 3-5% (CDC 2006a). The incubation time for *E. coli* O157:H7 is 2 to 5 d with an average duration of 8 d (FDA 2009a) and in most people, the disease is usually self-limiting in 5-10 d

(Tortorello 1999). The infectious dose has been reported to be as small as fewer than 50 bacterial cells (Tilden and others 1996), and all people are believed to be susceptible, but young children and the elderly appear to progress to more serious symptoms more frequently (FDA 2006a).

Epidemiological and Economic Impact of *E. coli* O157:H7

The prevalence of foodborne illnesses per year of *E. coli* O157:H7 has been estimated to be 73,480 illnesses each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (Mead and others 1999). From 1982 to 2002, a total of 350 outbreaks were reported from 49 states, accounting for 8,598 cases of *E. coli* O157 infection which included 1,493 (17.4%) hospitalizations, 354 (4.1%) cases of HUS, and 40 (0.5%) deaths (Rangel and others 2005). In 2008, the annual economic cost of illness caused by *E. coli* O157:H7 was \$478,061,302 with an average cost per case of \$6,506 (USDA 2009a). This estimate includes acute illness costs from hemorrhagic colitis and HUS as well as chronic illness costs arising from HUS with end-stage renal disease (USDA 2009a).

***E. coli* O157:H7 Outbreaks in Fresh Produce**

E. coli O157:H7 is not only associated with meat and meat products, though this organism is commonly shed in animal feces (Matthews 2006). Since more outbreaks of EHEC syndromes have been linked to beef than to any other single food source, it is believed that dairy herds are the primary reservoirs of these organisms (Jay and others 2005). Transmission of *E. coli* O157:H7 has also been spread through unpasteurized fruit juices, lettuce, spinach, contaminated drinking water, as well as through contact

with infected animals (such as in petting zoos) and person-to-person, especially among children in day care centers (CDC 2006a). Thus, produce is also a prominent transmission vehicle of this organism (Matthews 2006). Half of produce-associated outbreaks have been caused by kitchen-level cross-contamination and the other half were due to produce already contaminated with *E. coli* O157:H7 before purchase, including lettuce, sprouts, cabbage, apple cider, and apple juice (Rangel and others 2005). Produce items are thought to have become contaminated in the field from manure or contaminated irrigation water (Dean-Nystrom and others 1998; Rangel and others 2005).

From 1982 to 2002, produce-associated outbreaks of *E. coli* O157:H7 peaked in summer and fall; 74% occurred from July to October (Rangel and others 2005). Also, during the period of 1982 to 2002, produce was associated with 21% of foodborne outbreaks and 34% of 5,269 cases of foodborne *E. coli* O157:H7 illness (Matthews 2006). Thirteen (34%) produce associated outbreaks were from lettuce, 7 (18%) from unpasteurized apple cider or apple juice, 6 (16%) from salad, 4 (11%) from coleslaw, 4 (11%) from melons, 3 (8%) from sprouts, and 1 (3%) from grapes (Rangel and others 2005). The median number of cases in produce-associated outbreaks from 1982 to 2002 (20) was significantly larger than that of ground beef-associated outbreaks (8) ($p < 0.001$) (Rangel and others 2005). In 2006, a produce-borne outbreak linked to fresh spinach related to *E. coli* O157:H7 occurred across 26 states (Maki 2006; Lynch and others 2009) and is at least the 26th reported outbreak of *E. coli* O157:H7 infection that has been traced to contaminated leafy green vegetables since 1993 (Rangel and others 2005; Maki 2006). This outbreak affected 199 persons across the 26 states (CDC 2006a)

and *E. coli* O157:H7 was isolated from 13 opened packages of spinach provided by patients from 10 states; 11 of the packages had lot numbers indicating processing by a single manufacturing facility on the same day (Maki 2006).

***Salmonella* Classification and Growth Requirements**

One of the pathogens documented by the U.S. Food and Drug Administration (FDA) that has been isolated in the past from decaying fruits and vegetables is *Salmonella* (FDA 2009a). *Salmonella* belong to the family *Enterobacteriaceae*; these are Gram-negative, facultatively anaerobic enteric bacteria that are rod-shaped and are generally motile with peritrichous flagella, though non-motile strains/biovars have been identified (Bell and Kyriakides 2002). Most serovars ferment glucose with production of both acid and gas and do not ferment lactose (Cox 1999). Strains are catalase positive, cytochrome oxidase negative, indole negative, Voges-Proskauer negative, and methyl red positive and can utilize citrate as a sole carbon source (Bell and Kyriakides 2002; Cox 1999; D'Aoust 2000).

Salmonella are divided into two different species, *Salmonella bongori* and *Salmonella enterica* (Cox 1999; D'Aoust 2000; Bell and Kyriakides 2002). The species *S. enterica* is divided into six subspecies (*enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*) (D'Aoust 2000). These subspecies are divided into various serovars or serotypes within the Kauffmann-White antigenic scheme, based on differences in reaction with antibodies of two major and/or other minor types of cell-surface antigens (Bell and Kyriakides 2002; Cox 1999), and there are more than 2400 serotypes within the species of *S. enterica* of which 58.9% belong to the subspecies

enterica (D'Aoust 2000; Cox 1999; Bell and Kyriakides 2002). The Kauffmann-White Scheme for designation of *Salmonella* serotypes is maintained by the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella* at the Institut Pasteur and is used by most of the world (CDC 2006b).

For best growth, *Salmonella* require a pH between 6.6 and 8.2, and the minimum reported pH value for growth of *Salmonella* is 4.05 (Jay and others 2005). *Salmonella* are mesophilic and can grow within a temperature range of 2-54 °C, while temperatures below 7 °C have been observed only in bacteriological media but not in foods; growth at temperatures above 48 °C are confined to mutants and adapted strains (Cox 1999). The optimum temperature range for growth is 35-37 °C (D'Aoust 2000). Under optimum conditions the minimum water activity needed for the growth of *Salmonella* is 0.94 and the maximum needed is >0.99, yet *Salmonella* can survive in food products with a low water activity (Bell and Kyriakides 2002). Salmonellosis has also been associated with food products of low water activity such as some fermented meat products, hard cheese, peanut butter, chocolate, dried milk and cereal products and food ingredients such as black pepper and desiccated coconut (Bell and Kyriakides 2002).

Salmonellosis

The species that affects humans is *Salmonella enterica* which can cause the illness salmonellosis and organisms such as *S. Typhi*, *S. Paratyphi A*, *S. Paratyphi C* are agents of typhoid and paratyphoid fevers which are the most severe of the *Salmonella*-caused diseases (Jay and others 2005). In the *Salmonella* food-poisoning syndrome after the organism is ingested via food the organism will grow and multiply in their hosts'

body. Unlike other enteric pathogenic bacteria such as *Yersinia*, *Shigella*, and the enteroinvasive *E. coli* that replicate within the cytoplasm of host cells, *Salmonella* multiply within cellular vacuoles (D'Aoust 2000). *Salmonella* multiplies in the small intestine, colonizing and invading the intestinal tissues, producing an enterotoxin. The enterotoxin causes an inflammatory reaction (Bell and Kyriakides 2002) which can cause symptoms such as watery diarrhea, persistent and spiking fever, abdominal pain, headache, nausea, prostration, and a rash of rose-colored spots on the shoulders, thorax, or abdomen (D'Aoust 2000). Illnesses caused by *Salmonella* can range from gastroenteritis to enteric (typhoid) fever and septicemia and chronic sequelae (Bell and Kyriakides 2002). Septicemia is caused when *Salmonella* are present in the blood stream and is characterized by high fever, malaise, pain in the thorax and abdomen, chills, and anorexia (Bell and Kyriakides 2002). On the other hand, after effects are uncommon; some characteristics that have been identified include arthritis, osteoarthritis, appendicitis, endocarditis, and urinary tract infections (Bell and Kyriakides 2002). The typical incubation period for *Salmonella* is 18-72 h and symptoms can persist for 2-3 d (Jay and others 2005). The infectious dose of *Salmonella* has been reported to be as low as 10-100 cells (Bell and Kyriakides 2002). The average mortality rate is 4.1%, varying from 5.8% during the first year of life, to 2% between the 1st and 50th year, and 15% in persons over 50 years of age (Jay and others 2005).

Epidemiological and Economic Impact of *Salmonella*

Foodborne illnesses associated with *Salmonella* are estimated to be more than 1.3 million per year (Mead and others 1999). In 2006, 40,666 *Salmonella* isolates were

reported from participating public health laboratories via the National *Salmonella* Surveillance System, and 121 *Salmonella* outbreaks occurred, causing greater than 3,300 illnesses reported to the CDC Foodborne Outbreak Reporting System (CDC 2006b). In 2008, the estimated annual economic cost of illness caused by *Salmonella* was \$2,646,750,437 with an average cost per case of \$1,894 (USDA 2009a). This estimate is for all cases of Salmonellosis and includes medical costs due to illness, the cost of time lost from work due to nonfatal illness, and the cost of premature death (USDA 2009a).

***Salmonella* Outbreaks in Fresh Produce**

This organism can contaminate fruits and vegetables upon harvesting due to cross-contamination with livestock feces (Cox 1999). *Salmonella* are most prevalent in the intestinal tract of animals such as birds, reptiles, farm animals, humans, and occasionally insects; cells can be excreted in feces and be transmitted by insects and other living organisms to different places (Jay and others 2005). Recent produce-borne outbreaks include outbreaks from 2000-2002 of *Salmonella enterica* subsp. *enterica* serotype Poona associated with eating cantaloupe (Lynch and others 2009; Jay and others 2005; CDC 2002). One cantaloupe-transmitted outbreak of 2000-2001 affected 12 states (California, Washington, Oregon, Colorado, Nevada, Missouri, Texas, Arkansas, Minnesota, Vermont, New Mexico, and Arizona) and Canada (Jay and others 2005; CDC 2002). From April-June 2000, there were a total of 47 confirmed cases of *S. Poona* infections, and from April-May of 2001, there were 50 confirmed cases of *S. Poona* reported (CDC 2002). Also, from March-May 2002, there were a total of 58 confirmed cases of *S. Poona* reported (CDC 2002). In 2004, a multistate outbreak of *Salmonella*

enterica subsp. *enterica* serotype Javiana infections associated with tomatoes at a gas station deli chain affected more than 400 people in 5 states (CDC 2006b). Also in 2006, two *Salmonella* outbreaks were associated with consumption of raw tomatoes in restaurants. The first caused by *Salmonella enterica* subsp. *enterica* serotype Newport resulted in 119 illnesses in 18 states; the second was caused by *Salmonella enterica* subsp. *enterica* serotype Typhimurium and resulted in 190 cases across 21 states (CDC 2006b). In 2008, an outbreak of *Salmonella enterica* subsp. *enteric* serotype Saintpaul infections associated with jalapeño and serrano peppers occurred in 43 states, District Columbia, and Canada and over 1,440 persons reported being infected with the outbreak strain with patient ages ranging from less than 1 year in age to 99 years and the highest incidence among persons aged 20–29 years. (Lynch and others 2009; CDC 2008). Moreover, improper food-handling practices by consumers contribute to 40-60% of the cases of foodborne illness; thus, if consumers were able to take effective action in the domestic kitchen setting, this would in turn help reduce the incidence of foodborne illness and outbreaks (de Jong and others 2008).

CHAPTER III

CONSUMER IN-HOME PRACTICES AND CLEANING METHODS

Consumer Practices

In order to promote effective consumer food handling and sanitation, The Home Food Safety... It's in Your Hands (HFSYH) program was developed and implemented by the American Dietetic Association (ADA), ConAgra Foods Foundation, and FightBAC! (Cody and Hogue 2003). FightBAC! was developed and implemented by the Partnership for Food Safety Education (PFSE) in association with representatives from industry associations, professional societies in food science, nutrition and health consumer groups, the USDA, the Environmental Protection Agency (EPA), the Department of Health and Human Services (HHS), the CDC and FDA. A survey conducted by the HFSYH reported that 61% of consumers believe that inadequate cleaning and sanitation are the most common practices that might cause food poisoning and 82% responded that it is extremely important or very important to have information that helps them to take control of safety of foods prepared in their homes (Cody and Hogue 2003). It has also been reported that the main causes of foodborne illness include preparing food too far in advance and allowing growth of pathogens, improper cooling, inadequate reheating, and also cross-contamination via inanimate surfaces (Beumer and Kusumaningrum 2003).

The United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) recommends that the consumer not wash their produce with detergent or

soap because those products have not been approved or labeled by the FDA to be used on foods and could result in the ingestion of residues from the soap or detergent the produce might have absorbed (FSIS 2006; FDA 2009b), yet there are other options that can be used for the cleaning and disinfection of the surfaces of produce. A national survey was conducted among 2,000 randomly selected households by Li-Cohen and Bruhn (2002) in spring 2000 to assess consumer attitudes toward safety, handling and washing practices associated with fresh fruits and vegetables and received feedback from 624 consumers. When asked, 6% of consumers reported they seldom or never wash their produce, 19% reported washing before leaving produce out on a counter or in a bowl, 21% stated they washed produce before placing in the refrigerator, and 81% stated they washed produce just before preparing or cooking (Li-Cohen and Bruhn 2002). Reported washing methods included soaking in container or sink, washing under running water, use of vinegar, use of a dish detergent, use of a chlorine solution, or use of a commercial solution (Li-Cohen and Bruhn 2002). Consumers surveyed reported using vinegar, a chlorine solution, or dish detergent when washing fresh fruits and vegetables 1-4% of the time (Li-Cohen and Bruhn 2002). In a separate study, a panel of 80 consumers of at least 18 years of age and the primary shopper for the household, about 31% of respondents claimed they would be willing to buy an antibacterial solution for use in the home to treat lettuce prior to storage and/or consumption whereas 48.7% were undecided (McWatters and others 2001). A majority of respondents (82.5%) claimed that if they chose to use an antibacterial solution to treat lettuce at home that they preferred a ready-to-use, premixed solution, whereas 17.5% preferred a ready-to-mix concentrate for

dilution at the time of use (McWatters and others 2001). Sofos and others (1998) mentioned examples of antimicrobials of natural origin approved such as hydrogen peroxide and ethanol and the need to examine their efficacy and functionality in models of food systems and foods and mechanisms of action against microorganisms. Limited published scientific information exists regarding the effectiveness of household products against pathogens such as *Salmonella* and *E. coli* O157:H7 (Yang and others 2009). Although there is a strong focus on eliminating pathogens from produce at a commercial level, if given readily available and simple interventions, consumers might also achieve pathogen reduction from produce in a domestic setting. Moreover, consumers are generally in need of more practical information on how to reduce bacterial contamination on fresh produce (Kilonzo-Nthenge and others 2006).

Vinegar as a Consumer Available Antimicrobial

Organic acids such as acetic acid found in household distilled vinegar at a concentration of 5% have antimicrobial properties and are generally recognized as safe (GRAS) (Cherry 1999). According to FDA's Compliance Policy Guidance Manual Sec. 525.825, vinegars in the U.S. should contain no less than 4% acetic acid (1977). The critical steps in vinegar production are the preparation and the fermentation of raw materials as seen in Figure 4. Two steps are common to all vinegars: alcoholic and acetic acid fermentation, due to yeasts such as *Saccharomyces* spp. and acetic acid bacteria such as *Acetobacter* spp., respectively, while other microorganisms, such as molds and lactic acid bacteria, are involved only in specific types of vinegars. Fermentation can be

induced spontaneously, by back-slopping, or by the addition of starter cultures (Solieri and Giudici 2009).

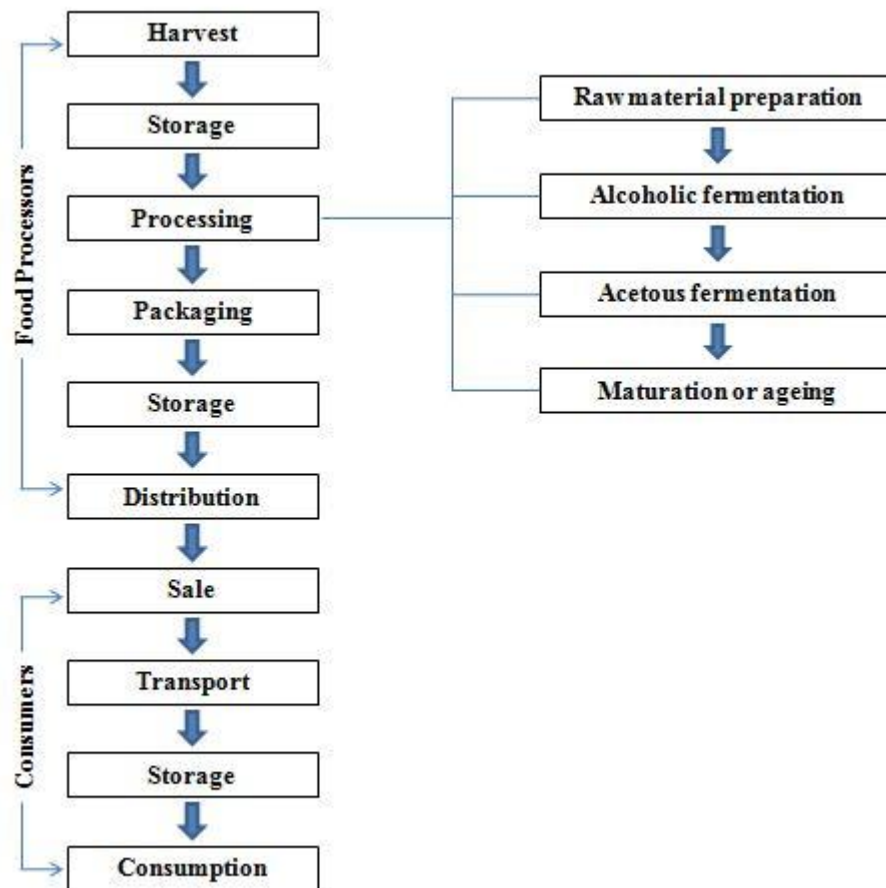


Figure 4 - Vinegar manufacture flow diagram. The steps in the production of vinegar from the processing to the consumer (Solieri and Giudici 2009).

The pH of commercial vinegars range from 2.40 and 3.20, and the acidity of commercial vinegars in the order of most acidic to least: white > rice > wine > raspberry wine > balsamic > grape > apple cider > apple (Shahidi and others 2008). The mode of action of organic acids is related to maintenance of acid-base equilibrium, proton

donation, and the production of energy by the cell (Davidson and Branen 1993). Antimicrobial activity of acetic acid depends upon exposure time, temperature, concentration of acid, dissociation level, and/or pH; effectiveness of acetic acid increases as its concentration increase, pH decreases, temperature increases, and microbial load decreases (Naidu 2000). The activity of organic acids is directly related to the concentration of undissociated molecules (protonated acids) which increase as pH decreases because of increasing amounts of protons, and the increased number of protons on the outer surfaces of microorganisms can disrupt membrane function by denaturing enzymes and by altering permeability leading to membrane destabilization (Naidu 2000). Entani and others (1998) determined the time necessary for vinegar containing 2.5% acetic acid to inactivate *E. coli* O157:H7 was 150 min; it was also determined that the number of cells in the inoculum of *E. coli* O157:H7 had almost no effect on the bactericidal activity of vinegar. A study by Segun and Karapinar (2005) showed vinegar could be used as a natural sanitizer in the home for the removal of *Salmonella* on spring onion. This study showed that spring onions exposed to vinegar containing 3.95% acetic acid after a 60 min treatment reduced *S. Typhimurium* 2.1 and 2.9 log₁₀ CFU/g with starting inoculums levels of 6.3 ± 0.4 log₁₀ CFU/g and 4.5 ± 1.1 log₁₀ CFU/g, respectively (Sengun and Karapinar 2005). Another study comparing the effectiveness of vinegar on shredded carrots inoculated at 5.7 log₁₀ CFU/ml showed that vinegar containing 4.03% acetic acid reduced *S. Typhimurium* 1.9, 2.6, and 3.6 log₁₀ CFU/g after 15, 30, and 60 min of exposure with no differences in log cycle reductions (p<0.05) at 15 and 30 min of exposure (Sengun and Karapinar 2004). Parnell and Harris

showed that applying 5 ml of vinegar containing 5% acetic acid, rubbing for 5 s, rinsing with 200 ml of water, and drying with a paper towel resulted in a reduction of approximately $6.2 \log_{10}$ CFU per apple (2003). Vijayakumar and Wolf-Hall (2002) using 35% white vinegar with 1.9% acetic acid reduced *E. coli* levels $5.0 \log_{10}$ CFU/g on lettuce after 5 min with agitation or after 10 min without agitation; sensory testing showed that samples treated with the white vinegar for 10 min were noticeably sour and wilted in appearance. Kilonzo-Nthenge and others (2006) showed that after soaking lettuce contaminated with *Listeria innocua* in a 5% vinegar solution for 2 min followed by a 15 s rinse in water, there was a reduction of $1.9 \log_{10}$ CFU/g. Chang and Fang (2007) examined the antimicrobial effect of rice vinegar on chopped lettuce with an initial inoculum of 4.0 and $7.0 \log_{10}$ CFU/g of *E. coli* O157:H7 at 25 °C. The study showed that commercial rice vinegar dilutions containing 0.05% acetic acid (pH 4.09) and 0.5% acetic acid (pH 3.26) reduced *E. coli* O157:H7 less than $1.0 \log_{10}$ CFU/g on chopped lettuce after an exposure of 5 min. Results from experiments using rice vinegar containing 5% (v/v) acetic acid on pathogen-inoculated lettuce samples showed that $7.0 \log_{10}$ CFU/g *E. coli* O157:H7 were reduced by 3.0 logs CFU/g after 5 min of treatment and low ($4.0 \log_{10}$ CFU/g) inoculum levels *E. coli* O157:H7 were reduced to an undetectable level ($<1 \log_{10}$ CFU/g) (Chang and Fang 2007). Medina and others (2007) examined the survival of foodborne pathogens in aqueous extracts of olive oil, virgin olive oil, vinegar, and several beverages. Vinegar at a pH 2.9 (5% acetic acid) reduced counts from an initial concentration of $6.0 \log_{10}$ CFU/ml of *Listeria monocytogenes*, *Salmonella* Enteritidis, *S. sonnei*, and *Yersinia* spp. to levels below the detection limit

and killed about 5.0 log CFU N_1/N_0 (N_0 is CFU per ml inoculated, N_1 is CFU per ml after 5 min of contact) *E. coli* and about 4.0 log CFU N_1/N_0 *Staphylococcus aureus* cells (Medina and others 2007).

Hydrogen Peroxide as a Consumer Available Antimicrobial

Hydrogen peroxide (H_2O_2) is a weak acid (Jay and others 2005), is GRAS, and not a hazard to the public when used at levels that are now currently being used in-home by consumers (FDA 2006b). Hydrogen peroxide decomposes rapidly into water and oxygen leaving no residual toxicity; its bactericidal activity is well characterized (Cherry 1999). Hydrogen peroxide does not act as an antimicrobial except by the production of a reactive oxygen species (ROS)/radical such as singlet or superoxide oxygen (higher energy form of oxygen); superoxide can cause oxidative destruction of lipids and other macromolecules (DNA, proteins/enzymes) (Davidson and Branen 1993). Studies on the effects of 2.5% and 5% H_2O_2 treatment on fresh-cut and whole cantaloupe and honeydew contaminated with *Salmonella* spp. showed a significant reduction of approximately 3.0 log₁₀ CFU/cm² on whole honeydew and 2.0 log₁₀ CFU/cm² on whole cantaloupe but no significant impact on bacterial inhibition between 2.5% and 5% concentrations (Ukuku 2004). For the fresh-cut cantaloupe, both 2.5% and 5% concentrations yielded a reduction of *Salmonella* of 2.0 log₁₀ CFU/g, and the fresh-cut honeydew yielded a reduction of 1.3 log₁₀ CFU/g (Ukuku 2004). Sapers and others reported the effects of rinsing on the efficacy of H_2O_2 -based wash for decontamination of Golden Delicious apple halves inoculated with non-pathogenic *E. coli*; the results showed 2.3 log₁₀ CFU/g reduction of *E. coli* after rinsing for 1 min at 50 °C with 5%

H₂O₂ (2000). Samadi and others (2009) demonstrated that 133 ppm H₂O₂ for 30 min and 100 ppm peroxyacetic acid for 15 min reduced total mesophilic microbial counts by 2.8 log CFU/g on surfaces of various produce items (radish, parsley, basil, cilantro, leek, and peppermint).

Ethanol as a Consumer Available Antimicrobial

Ethanol (ethyl alcohol) is a component in alcoholic beverages and can be produced through microbial fermentation. The fermentation is generally composed of three steps: the formation of solution of fermentable sugars, the fermentation of these sugars to ethanol via bacterial or fungal microbes, and the separation and purification of the ethanol, usually by distillation (Janick and Whipkey 2002). Ethanol has been used as an antimicrobial since the first alcoholic fermentation was practiced to preserve fruit by denaturing proteins in the cytoplasm and is bactericidal at high concentrations (Davidson and Branen 1993). Ethanol has been found to be effective in reducing pathogens on the surface of produce (Beier and others 2004). Studies using an ethanol dip for the removal of *E. coli* from the surface of grapes showed that at a concentration of 50%, a 3 min exposure time resulted in a reduction of approximately 3.0 log₁₀ CFU/g; results were highly variable with 10 of the 48 trials resulting in less than 1 log₁₀ CFU/g reduction (Pinto and others 2006). Medina and others (2007) examined the survival of foodborne pathogens in beer with 5% alcohol at pH 4.6, alcohol-free beer at pH 4.3, red wine at pH 3.7 with 13% alcohol, and white wine at pH 3.3 with 11.5% alcohol. In this study, a 1.4 log₁₀ CFU/ml reduction was observed in beer containing 5% alcohol inoculated with one of two *Salmonella* Enteritidis strains (Medina and others 2007). Just and Daeschel

(2003) studied the survival of *E. coli* O157:H7 and *Salmonella* spp. in grape juice and wine at room temperature (Chardonnay at 13.6% alcohol and Pinot Noir at 15.3% alcohol), and determined that pathogens (at an initial concentration of 6.3-6.9 log CFU/ml) were inactivated in wine within 60 min but survived up to 16 d in the grape juice.

Consumer Methods of Knife Handling

Cross-contamination plays an important role in the transmission of food-borne pathogenic microbes, especially when consumers are preparing dishes in the home (de Jong and others 2008; van Asselt and others 2008). Microorganisms can diffuse and spread from contaminated foods, such as raw poultry, fish or meat, to hand and food contact surfaces in the domestic kitchen (for example, cutting boards, knives) (Gorman and others 2002). Improper food-handling practices by consumers contribute to 40-60% of the cases of foodborne illness, and improved consumer hygiene may be an important factor in the reduction of food-borne gastroenteritis (de Jong and others 2008). van Asselt and others (2009) conducted a study video-taping 25 participants in their preparation of a chicken salad to observe cross-contamination; only 29% of the volunteers were able to prevent cross-contamination. Consumer-reported methods of handling knives after handling meat and before cutting fresh produce include: use of a different knife, washing of knives with dishwashing liquid, rinsing with water, wiping with paper towel (Li-Cohen and Bruhn 2002). Surfaces from which it is easier to remove food debris and microorganisms are generally considered the most hygienic; however, the characteristics that enable a surface to be easily cleaned (and more easily

microbiologically sampled) may also render it more likely to release organisms during food contact (Moore and others 2007).

The retention of bacteria on food contact surfaces increases the risk of cross-contamination of these microorganisms to food (Kusumaningrum and others 2003). Wilks and others (2005) inoculated different metal surfaces with *E. coli* O157 and measured the survival time, showing that *E. coli* O157 can survive for >28 d of refrigerated (4 °C) and room temperature (20 °C) storage on stainless steel surfaces. Ortega and others (2010) showed that adhesion of *E. coli* to stainless steel surface took 2-4 h of exposure to reach a plateau of surface cell density, and after 15 min of whirlpool rinsing, the surface cell density showed a decrease of around 65% with peptone saline solution as a rinsing medium and a 99% decrease by rinsing with water. Moore and others (2003) conducted a study investigating transfer of *S. Typhimurium* to dry lettuce and showed that there was an increase from 36-66% in the transfer of the initial inoculum during the first 60 min of sampling, followed by a steep drop percent transfer; transfer of *S. Typhimurium* to wet lettuce ranged from 23 to 31%. Moore and others (2007) studied the recovery of *S. Typhimurium* on different kitchen surfaces, wood, stainless steel, Formica, and polypropylene; in most cases, a significantly higher number of bacteria were recovered from Formica and stainless steel recovered compared with polypropylene or wood surfaces over the 6 h period. Kusumaningrum and others (2003) hypothesized that the risk of cross-contamination is lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced, though it was also hypothesized that some non-sporeforming bacteria might be able to withstand dry

conditions on surfaces for an extensive period of time . The study showed that *S. Enteritidis* was recovered from stainless steel surfaces for at least 4 d at high contamination levels, but at moderate level, the numbers decreased to the detection limit within 24 h. Thus, pathogens may remain viable on dry stainless steel surfaces and present a contamination hazard for considerable periods of time, dependent on the contamination levels and type of pathogen (Kusumaningrum and others 2003). Mattick and others (2003) examined the risk of bacterial transfer onto sterile dishes and sponges via contaminated water, kitchen surfaces wiped with a contaminated sponge, items placed in direct contact with a contaminated kitchen surface, food placed on a contaminated dish or dishes from contaminated food. This study showed that *E. coli* and *Salmonella* survived towel- or air-drying on dishes and after towel-drying the cloth became contaminated on every occasion, regardless of the test organism. Also, a proportion of sterile dishes washed after contaminated dishes became contaminated with pathogens but transfer from dishes onto food was rare. Finally, the study showed that washing-up sponges frequently became contaminated with pathogens (Mattick and others 2003). Goulter and others (2008) determined the effect of combinations of time and temperature ranging from 1 s to 60 s and 60 °C to 82 °C on the disinfection of knives artificially contaminated with *E. coli*. The lowest reduction in counts achieved was $<0.5 \log_{10}$ CFU/ml (1 s; 60, 70, and 75 °C), and the greatest was $5.3 \log_{10}$ CFU/ml (60 sec; 80 °C). At all temperatures, increasing the length of immersion from 1 to 5 s gave a significant increase in bacterial reduction ($p < 0.01$), and for temperatures of 70

°C and above, increasing the immersion time from 5 to 10 s also significantly increased the bacterial reduction ($p < 0.01$) (Goulter and others 2008).

The purpose of this study was to determine the efficacy of consumer-available food antimicrobials H₂O₂, acetic acid, and EtOH to disinfect surfaces of waxed green bell peppers artificially contaminated with *S. enterica* serovars and *E. coli* O157:H7 under conditions similar to those likely applied in a domestic kitchen setting. This study also aimed to determine the efficacy of consumer cleaning methods for the decontamination of knives used to chop the contaminated bell peppers, and assess the potential for transfer of *S. enterica* serovars and *E. coli* O157:H7 on the surfaces of artificially contaminated green bell peppers to a non-treated produce item (salad cucumbers) via contaminated knives. Prior to these studies, preliminary trials were conducted to achieve an adequate initial inoculum level to attain a high concentration of cells on the surface of the bell pepper. Two different inoculation methods were tested to determine the most efficient method for the studies, spot inoculation and dip inoculation. A validation of chopping sizes was conducted to assess which chopping style would achieve the highest attachment of pathogens to the knife blade. A preliminary study was also conducted to determine which sampling method of the knife blade, swab or sponge, would be most effective for sampling.

CHAPTER IV

MATERIALS AND METHODS

Bacterial Culture Preparation and Maintenance

Rifampicin-resistant strains of *Salmonella enterica* serovars Montevideo and Poona, *Salmonella* Typhimurium American Type Culture Collection 13311 (ATCC, Manassas, Va.), and *E. coli* O157:H7 (designated P41, P8, and E34; beef cattle carcass isolates) were obtained from the Department of Animal Science Center for Food Safety culture collection at Texas A&M University (College Station, Tex.). Cultures were maintained on tryptic soy agar (TSA; Becton Dickinson and Co., Sparks, Md.) slants at 5 °C. Working cultures were obtained by transferring a loop of culture from TSA slants to 10 ml of tryptic soy broth (TSB; Becton Dickinson and Co.) and incubating aerobically with agitation at 35 °C for 24 h. API20E[®] (bioMérieux USA, Hazelwood, Mo.) testing was conducted according to manufacturer instructions to biochemically confirm species of the microorganisms.

Preliminary Experiments

Dip Inoculation Validation. Waxed green bell peppers were purchased at a local grocery store in College Station, Tex. and transported to the laboratory the day of purchase. *Salmonella* and *E. coli* O157:H7 strains were inoculated from TSA slants into 10 ml of TSB and incubated at 35 °C for 24 h as described above. After 24 h, a loop of each strain was transferred to fresh TSB and incubated at 35 °C for 24 h. After incubation, 10 ml of each culture was transferred to sterile 15 ml conical centrifuge tubes

(Thermo-Fisher Scientific, Waltham, Mass.). The suspension then was washed by centrifugation at 2191 x g in a Jouan B4i centrifuge (Thermo-Fisher Scientific) for 15 min at 22 °C. Bacterial pellets were re-suspended in 10 ml of 0.1% Peptone (Becton Dickinson and Co.) and washed; centrifugation was repeated identically twice for 15 min at 22 °C. The pellets were re-suspended in 10 ml of 0.1% Peptone, and 5 ml aliquots of each strain was combined to make a cocktail in a sterile Erlenmeyer flask containing 2970 ml 0.1% (w/v) Peptone. Inoculation of peppers was carried out by dipping the pepper in a sterile beaker containing the bacterial cocktail for different lengths of time (1, 2, 3, 4, 5, 10, 15, 30, and 60 min) to determine the time lapse resulting in greatest bacterial attachment. The peppers were then drip-dried for 60 min at room temperature. Following inoculation, samples consisting of 3-10 cm² excisions/sample were taken using flame-sterilized scalpels and forceps and composited in a stomacher bag. Ninety-nine ml of 0.1% Peptone were added and the bag was stomached (230 rpm) for 1 min. Serial dilutions were prepared with 0.1% Peptone. Two non-inoculated control samples were processed/enumerated on Plate Count Agar (PCA; Becton Dickinson Co.) to quantify the aerobic plate count (APC). Lactic acid bacteria (LAB) were enumerated using deMann, Rogosa, and Sharpe (MRS) agar (Becton Dickinson Co.). Yeasts and molds were selectively plated on Potato Dextrose Agar (PDA; Becton Dickinson Co.) acidified to pH 3.5 with 10% Tartaric Acid (Becton Dickinson Co.). *Salmonella* and *E. coli* O157:H7 were enumerated on lactose-sulfite-phenol red-rifampicin agar (LSPR), and plates of LSPR were incubated aerobically for 24 h at 35 °C prior to survivor enumeration (Castillo and others 1998). PCA plates were incubated aerobically for 48 h

at 35 °C, and plates of MRS were incubated anaerobically for 48 h at 35 °C prior to survivor enumeration. Plates of PDA were incubated aerobically for 7 days at 22 °C prior to survivor enumeration.

Spot Inoculation Validation. Bacterial cocktail was prepared as described in the Dip Inoculation Validation. The pellets were re-suspended in 10 ml of 0.1% Peptone, and 1 ml aliquots of each strain was combined to make a cocktail in a sterile Erlenmeyer flask containing 594 ml 0.1% peptone. Prior to cocktail preparation, 1 ml from each centrifuge tube for each strain was transferred to 1-15 ml tube. From this tube, dilutions 10^6 - 10^8 were made in both TSB and 0.1% Peptone to create $5.0 \log_{10}$ CFU/ml, $6.0 \log_{10}$ CFU/ml, and $7.0 \log_{10}$ CFU/ml inoculums. Twenty samples were prepared by excising a 30-cm² sample from a bell pepper and placing the excised piece in a sterile Petri dish. Two samples were made per pepper, one for a 1 h dry time and another for a 24 h dry time. Each sample was spotted with 10- 10 μ L of designated inoculum (cocktail A-B, 10^6 - 10^8 of either 0.1% Peptone or TSB, and non-inoculated TSB or 0.1% Peptone). Samples were either left to dry for 1 h or 24 hr. Procedure of spotting was carried out in a bio-safety cabinet (Nuair 425-300, Plymouth, Maine). After drying, samples were placed in a stomacher bag. Ninety-nine ml of 0.1% Peptone were added and the bag was stomached (230 rpm) for 1 min. Dilution blanks consisted of 9 ml of 0.1% Peptone. Two non-inoculated control samples were enumerated on PCA for total aerobic plate count. Lactic acid bacteria were enumerated using MRS agar. Yeasts and molds were selectively plated on PDA. Plates of LSPR were incubated aerobically for 24 h at 35 °C prior to survivor enumeration. PCA plates were incubated aerobically for 48 h at 35 °C,

and plates of MRS were incubated anaerobically for 48 h at 35 °C prior to survivor enumeration. Plates of PDA were incubated aerobically for 7 days at 22 °C prior to survivor enumeration.

Bacterial Lawn Method Validation. Waxed green bell peppers were purchased at a local grocery store in College Station, TX and transported to the laboratory the day of purchase. *Salmonella* serovars and *E. coli* O157:H7 were inoculated from TSA slants into 10 ml of TSB and incubated at 35 °C for 24 h as described above. Next, 2 ml of culture broth was transferred to 225 cm² surfaces of sterile TSA in bottles; each strain was inoculated individually into a separate bottle. The inoculum was spread throughout the TSA surface by aseptically adding approximately 80, 5 mm-diameter, sterile glass beads (Thermo-Fisher Scientific) and rotating the beads at a rate of 1 rotation per s for 10 rotations over the entire surface of the agar (Danyluk and others 2005). Inoculated bottles were incubated at 35 °C for 24 h to obtain a bacterial lawn. Growth from each culture bottle was harvested by adding 10 ml of 0.1% Peptone to each bottle and swirling the glass beads left from the inoculation step and transferred with a pipette to sterile 15 ml conical centrifuge tubes. Centrifugation procedures were followed from the previous validations. Inoculation of peppers was carried out by dipping the pepper in a sterile beaker containing the bacterial cocktail for different time points (1 and 5 min) to confirm that there was no difference between time points. Sampling and plating procedures from the Dip Inoculation Validation were followed.

Swab and Sponge Validation. Cocktails were prepared using the methods outlined in the Dip Inoculation Validation procedure. Three 60 ml cocktails were made

using TSB to achieve $5.0 \log_{10}$ CFU/ml, $4.0 \log_{10}$ CFU/ml, and $3.0 \log_{10}$ CFU/ml. Fifty ml of bacterial cocktail was added to a sterile 50 ml graduated cylinder. Knives and cutting board were sterilized by autoclaving at $121 \text{ }^{\circ}\text{C}$ for 15 min. A sterile knife was dipped into a graduated cylinder containing the cocktail for 1 min. The knife was then dried for 30 min. After 30 min, one knife was sampled with a sponge (10 times on each side of the blade) and 1 knife was sampled with a swab, 1 swab per side of blade, totaling 2 swabs per knife. The swabs were moistened in 10 ml of 0.1% Peptone, and the sponge was hydrated with 10 ml of Buffered Peptone. Samples were enumerated on LSPR and dilutions created using 9 ml 0.1% Peptone. Plates of LSPR were incubated aerobically for 24 h at $35 \text{ }^{\circ}\text{C}$ prior to survivor enumeration.

Chopping Validation. Cocktail preparation was followed according to the outlined procedure in the Bacterial Lawn Method Validation. After peppers dried for 60 min, peppers were chopped by first removing the stem area, followed by preparation of lengthwise latitudinal slices at a width of 1 cm. Next, the pepper was chopped into squares with a surface area of either 2 cm^2 (Large), 1 cm^2 (Medium), or 0.5 cm^2 (Small). After chopping, the knives were sampled using a swab, 1 swab per side of knife blade, totaling 2 swabs per knife. The swabs were moistened in 10 ml of 0.1% Peptone. Prior to and following inoculation of the peppers, pepper samples consisting of 3 10-cm^2 excisions/sample were taken using flame-sterilized scalpels. Ninety-nine ml of 0.1% Peptone were added to these samples and the bag was stomached (230 rpm) for 1 min. Dilution blanks consisted of 9 ml of 0.1% Peptone. Both swab samples and pepper samples were enumerated on LSPR and dilutions created using 9 ml 0.1% Peptone.

Plates of LSPR were incubated aerobically for 24 h at 35 °C prior to survivor enumeration.

Study 1: Antimicrobial Disinfection of Pepper Surfaces

Waxed green bell peppers were purchased at a local grocery store in College Station, Tex. and transported to the laboratory the day of purchase. Inoculation preparation was carried out according to the procedure outlined in the Bacterial Lawn Validation Preliminary Experiment. The pellets were suspended in 10 ml of 0.1% Peptone water, and 10 ml aliquots of each strain were combined to make a cocktail in a sterile Erlenmeyer flask containing 540 ml 0.1% Peptone water resulting in a concentration of $8.8 \pm 0.1 \log_{10}$ CFU/ml of *Salmonella* strains and $8.6 \pm 0.2 \log_{10}$ CFU/ml of *E. coli* O157:H7 strains based on preliminary experiments (Danyluk and others 2005). Inoculation of peppers was carried out by dipping the pepper in a sterile beaker containing the bacterial cocktail for 1 min. The peppers were then drip-dried for 60 min at room temperature. Following inoculation, samples consisting of 3 10-cm² excisions per sample were taken using flame-sterilized scalpels. Two samples were placed in stomacher bags without being immersed in an antimicrobial. Ninety-nine ml of 0.1% Peptone were added and the bag was stomached (230 rpm) for 1 min. Dilution blanks consisted of 9 ml of 0.1% Peptone. These samples were enumerated to determine the bacterial load on peppers after inoculation and prior to treatment. The remaining pepper samples were used for treatment with an antimicrobial. Two samples were immersed for 0.25, 0.5, 1, 2, 3, 4, or 5 min in a solution of 3% Hydrogen Peroxide (v/v) (Wal-Mart, Bentonville, Ark.) 2.5% Acetic Acid (v/v) (H.J. Heinz Co. Distilled White

Vinegar, Pittsburg, Pa.), 70% EtOH (v/v), or sterile distilled water (SDW). After each sample was immersed in a treatment, the samples were immersed for 30 s in a neutralizer solution prepared as outlined by Black and others (2008). Neutralizer solution for EtOH and acetic acid-exposed samples were prepared by first making a concentrate consisting of 40 g lecithin (Sigma-Aldrich Co., St. Louis, Mo.), 1.25 ml of phosphate buffer stock (8 g NaCl, 0.2 g Na₂HPO₄, 1.15 g Na₂HPO₄, 0.2 g KCl and diluted with Distilled H₂O to 1 L) (Sigma-Aldrich Co.) and 280 ml Polysorbate 80 (Tween 80; Sigma-Aldrich Co.) diluted with SDW to 1 L and adjusted to pH 7.2 with 1.0 N NaOH. This neutralizer concentrate was used to make the neutralizer solution by adding 100 ml of concentrate to 25 ml of 0.25 M phosphate buffer stock and 1675 ml SDW. The solution was sterilized by autoclaving at 121 °C for 15 min. In the case of hydrogen peroxide-treated samples, a neutralizer consisting of 10,000 IU/ml *Micrococcus lysodeikticus*-fermented catalase (Sigma-Aldrich Co.) was prepared in SDW and filter sterilized through a 0.2 µm-pore cellulose acetate membrane filter (Corning Inc., Corning, N.Y.) (Black and others 2008). Neutralizers were used to inactivate antimicrobials after treatment. The samples were then placed in stomacher bags, and 99 ml of 0.1% Peptone was added. The bag was stomached for 1 min. Samples were enumerated on LSPR and dilutions created using 9 ml 0.1% Peptone. Two non-inoculated control samples were used to enumerate aerobic microbes on PCA as described previously. Lactic acid bacteria were enumerated using MRS agar; yeasts and molds were selectively plated on PDA as described previously. Plates of LSPR were incubated aerobically for 24 h at 35 °C prior to survivor enumeration. PCA plates were

incubated aerobically for 48 h at 35 °C, and plates of MRS were incubated anaerobically for 48 h at 35 °C prior to survivor enumeration. Plates of PDA were incubated aerobically for 7 d at 22 °C prior to survivor enumeration.

Study 2A: Efficacy of Decontamination of Knives

Inoculum preparation and inoculation of bell peppers were carried out as described in Study 1. Knives and cutting board were sterilized by autoclaving at 121 °C for 15 min. After peppers dried for 60 min, peppers were chopped by first removing the stem area, followed by preparation of lengthwise latitudinal slices at a width of 1 cm. Next, the pepper was chopped into squares with a surface area of approximately 1 cm². Knives were then treated with one of five cleaning methods: control (no treatment), a towel wipe and dry exposure, running hot water for 5 s, running hot water for 10 s and 1% detergent solution (v/v) of Dawn Ultra Concentrated, Original Scent (Proctor & Gamble, Inc., Cincinnati, Ohio) followed by hot running water for 10 s. The towel wipe and dry exposure consisted of a sterile, 100% Cotton, towel purchased at a local retail grocery store. The towel was cut to a surface area of 55-cm² and sterilized by autoclaving at 121 °C for 15 min. The towel was folded around the non-edged blade and quickly wiped one time. For the running hot water, the hot water was set at 60 °C and knives held under the running water for 5 s and 10 s. For the detergent treatment, a sterile sponge was hydrated with the soap solution and used to wipe the knife blade 3 times on each side. After each knife was exposed to a treatment, the blade surface was sampled with a sterile cotton swab hydrated with 0.1% Peptone for each side of the blade of the knife, totaling 2 swabs per knife. Swabs were placed in 10 ml of 0.1%

Peptone and mixed. Survivors were enumerated on LSPR. Enrichment broth was prepared by using Nutrient Broth (NB; Becton Dickinson) with the addition of rifampicin (Sigma-Aldrich Co.) at 0.1 g/ L of medium after autoclaving. From each swab sample, 1 ml was added to 9 ml of enrichment broth and dilutions were created using 0.1% Peptone and plated on LSPR. All plates were incubated aerobically for 24 h at 35 °C prior to survivor enumeration. The enrichment broth was incubated aerobically for 24 h at 35 °C without shaking in order to determine presence of growth/turbidity, indicative of transfer of pathogens at levels below the detection limit of plating assays. If growth was observed, a loop of enrichment medium was streaked on Xylose Lysine Desoxycholate agar (XLD; Becton Dickinson Co.) and incubated aerobically for 48 h at 35 °C to confirm presence of *Salmonella* and *E. coli* O157:H7.

Study 2B: Transfer Testing

Inoculum preparation and inoculation of bell peppers was conducted as previously outlined in Study 2A. Sterile knives were used to chop peppers as previously outlined, and each knife was treated by 1 of the previously outlined treatments. The knives were then used to chop a cucumber that was perforated using a sterile metal ruler to 2-mm thick slices. After the chopping of the cucumber, an approximate 11 g sample consisting of 2 cucumber slices with an approximate surface area of 80 cm² were weighed in a stomacher bag, and 99 ml of 0.1% Peptone was then added to the bag. The bag was stomached for 1 min and samples were enumerated on LSPR. Plates were incubated aerobically for 24 h at 35 °C prior to survivor enumeration. Enrichment broth was prepared as described above using NB with rifampicin (0.1 g/L). From each

stomached cucumber sample, 10 ml was added to 90 ml of enrichment broth and dilutions were created using 0.1% Peptone and plated on LSPR. The enrichment broth was incubated aerobically for 24 h at 35 °C without agitation in order to observe presence of growth. If growth was observed, a loopfull was streaked on XLD agar and incubated aerobically for 48 h at 35 °C to confirm presence of *Salmonella* and *E. coli* O157:H7.

Statistical Analysis

All experiments were replicated three times with duplicate samples, and microbiological data (plate counts) were transformed logarithmically (base 10) before statistical analysis. All analyses were conducted using JMP v8.0.1 (SAS Institute Inc., Cary, N.C.). For the antimicrobial testing, statistical differences between mean log reductions for each combination of antimicrobial and time exposure was determined and analyzed using a one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) ($p < 0.05$). For the decontamination of the knives, statistical differences between mean log reduction of each treatment was determined and analyzed using ANOVA and Fisher's LSD ($p < 0.05$), and for the transfer testing, differences between means of each treatment effect on the transfer to the cucumber was analyzed using ANOVA and Fisher's LSD ($p < 0.05$).

CHAPTER V

RESULTS

Preliminary Studies

Dip Inoculation Validation. Cocktails of rifampicin-resistant *Salmonella* and *E. coli* O157:H7 were dip inoculated onto waxed green bell peppers at a concentration of approximately of $6.8 \pm 0.1 \log_{10}$ CFU/cm² *Salmonella* and $6.8 \pm 0.0 \log_{10}$ CFU/cm² *E. coli* O157:H7. Duplicate samples consisting of 3 10-cm² pieces of inoculated pepper were excised from smooth tissue and immersed for 0, 1, 2, 3, 4, 5, 10, 15, 30, or 60 min. Statistical analysis indicated that there was no difference in attachment of the bacteria to the surfaces of bell peppers as a result of increased inoculation time (Table 2).

Table 2- Bacterial attachment to bell pepper by dipping at various time points.^a

Dip Time (min)	<i>Salmonella</i> (log ₁₀ CFU/cm ²)	<i>E. coli</i> O157:H7 (log ₁₀ CFU/cm ²)
1	2.7 ± 0.5 ^b	2.4 ± 0.9 ^b
2	2.8 ± 0.5 ^{ab}	2.9 ± 0.6 ^{ab}
3	3.4 ± 1.0 ^{ab}	3.4 ± 0.9 ^a
4	2.9 ± 1.0 ^{ab}	2.8 ± 1.1 ^{ab}
5	3.7 ± 0.5 ^a	3.7 ± 0.5 ^a
10	3.2 ± 0.7 ^{ab}	3.1 ± 0.7 ^{ab}
15	3.0 ± 0.6 ^{ab}	3.0 ± 0.6 ^{ab}
30	3.0 ± 0.5 ^{ab}	3.1 ± 0.6 ^{ab}
60	3.7 ± 1.3 ^{ab}	3.6 ± 1.2 ^a

^aValues represent means of triplicate replications with mean separation by Fisher's LSD; means within the same column and box with the same letter are not significantly different ($p \geq 0.05$).

Spot Inoculation Validation. A cocktail of rifampicin-resistant *Salmonella* and *E. coli* O157:H7 was prepared at a concentration of $6.4 \pm 0.5 \log_{10}$ CFU/cm² *Salmonella* and $6.2 \pm 0.5 \log_{10}$ CFU/cm² *E. coli* O157:H7. Prior to cocktail preparation, 1 ml from each centrifuge tube for each strain was transferred to a single 15 ml tube. From this tube, dilutions 10^6 - 10^8 were made in both TSB and 0.1% Peptone to create $5.0 \log_{10}$ CFU/ml, $6.0 \log_{10}$ CFU/ml, and $7.0 \log_{10}$ CFU/ml inoculums. Twenty samples were prepared by excising a 30-cm² sample from a bell pepper and placing the excised piece in a sterile Petri dish. Two samples were made per pepper, one for a 1 h dry time and another for a 24 h dry time. Each sample was spotted with 10 10- μ L aliquots of designated inoculum. Samples were either left to dry for 1 h or 24 h (Table 3). Statistical analysis determined no difference with respect to the attachment to the surface between the 1 h and 24 h dry times. There was also no significant difference between preparing the inoculums using TSB or 0.1% Peptone in respect to the attachment of pathogens to the surface of the bell pepper.

Table 3- Spot inoculation validation results.^a

Dry Time (h)	Inoculum (log ₁₀ CFU/ml) ^b	<i>Salmonella</i> (log ₁₀ CFU/cm ²) ^b	<i>E. coli</i> O157:H7 (log ₁₀ CFU/cm ²) ^b
1	5.0 Peptone	3.9 ± 0.4 ^c	4.0 ± 0.1 ^c
	6.0 Peptone	5.0 ± 0.3 ^{ab}	5.1 ± 0.3 ^{ab}
	7.0 Peptone	5.6 ± 0.3 ^a	5.7 ± 0.3 ^a
	5.0 TSB	4.0 ± 0.4 ^{ac}	4.0 ± 0.2 ^c
	6.0 TSB	4.8 ± 0.3 ^{abc}	4.2 ± 1.0 ^{bc}
	7.0 TSB	5.8 ± 0.4 ^a	5.7 ± 0.3 ^a
	24	5.0 Peptone	4.9 ± 1.1 ^{bc}
6.0 Peptone		5.2 ± 0.3 ^{abc}	5.0 ± 0.7 ^{bc}
7.0 Peptone		5.5 ± 0.4 ^{abc}	5.2 ± 0.9 ^b
5.0 in TSB		6.6 ± 0.4 ^{ab}	6.3 ± 0.4 ^{ab}
6.0 in TSB		6.8 ± 0.3 ^{ab}	6.3 ± 0.2 ^{ab}
7.0 in TSB		7.0 ± 0.4 ^a	6.9 ± 0.3 ^a

^aValues represent means of triplicate replications with mean separation by Fisher's LSD; means within the same column and box with the same letter are not significantly different ($p \geq 0.05$).

^bTable represents the means on surface of bell pepper according to the inoculums used. Inoculums were made in either 0.1% Peptone or TSB as indicated. Samples were left to dry for either 1 h or 24 h.

Bacterial Lawn Method Validation. A cocktail of rifampicin-resistant

Salmonella and *E. coli* O157:H7 was dip inoculated on to waxed green bell peppers at an inoculum concentration of approximately of $8.8 \pm 0.1 \log_{10}$ CFU/cm² *Salmonella* and $8.6 \pm 0.2 \log_{10}$ CFU/cm² *E. coli* O157:H7. Duplicate samples consisting of 3 10-cm² pieces of inoculated pepper were excised from smooth tissue and immersed the inoculums for 0, 1 and 5 min. Statistical analysis indicated there was no difference between attachment of the bacteria to the surface of the bell pepper at the various time points. The bacterial lawn method created a higher starting concentration for the bacterial cocktail. After a 1 min dip time and 60 min dry time, the concentration on the surface was $5.2 \pm 0.6 \log_{10}$ CFU/cm² and $5.1 \pm 0.6 \log_{10}$ CFU/cm² for *Salmonella* and *E. coli* O157:H7 respectively.

After a 5 min dip time and 60 min dry time, the concentration on the surface was $4.9 \pm 0.6 \log_{10}$ CFU/cm² and $4.8 \pm 0.7 \log_{10}$ CFU/cm² for *Salmonella* and *E. coli* O157:H7, respectively. These values represent means of triplicate replications plus or minus one standard deviation from the mean.

Sponge and Swab Validation. Three cocktails of rifampicin-resistant *Salmonella* and *E. coli* O157:H7 strains were made using TSB to achieve cocktails of $5.0 \log_{10}$ CFU/ml, $4.0 \log_{10}$ CFU/ml, and $3.0 \log_{10}$ CFU/ml concentrations. Fifty ml of bacterial cocktail was added to a sterile 50 ml graduated cylinder. A sterile knife was dipped in the graduated cylinder containing the cocktail for 1 min. The knife was then dried for 30 min. After 30 min, one knife was sampled with a sponge (10 times on each side of the blade) and 1 knife was sampled with a swab, 1 swab per side of blade, totaling 2 swabs per knife (Table 4). Statistical analysis determined that there was no significant difference between the use of the swab and the use of the sponge at the inoculum concentrations for the knife sampling.

Table 4- Sponge and swab validation results.^a

	Inoculum (log₁₀ CFU/ml)	Swab (log₁₀ CFU/38 cm²)	Sponge (log₁₀ CFU/38 cm²)
<i>Salmonella</i>	4.7 ± 0.0	3.4 ± 0.0	3.1 ± 0.5
	3.7 ± 0.1	2.4 ± 0.4	2.6 ± 0.2
	2.7 ± 0.0	Below Detectable Limit ^b	0.7 ± 0.9
<i>E. coli</i> O157:H7	4.6 ± 0.1	3.4 ± 0.0	3.3 ± 0.8
	3.7 ± 0.0	2.4 ± 0.4	2.7 ± 0.3
	2.5 ± 0.3	Below Detectable Limit ^b	0.7 ± 0.9

^aValues represent means of triplicate replications of cells recovered from the knife blade via either the sampling method of swab or sampling method of sponge.

^bDetectable limit was 1 CFU/ml.

Chopping Validation. Inoculum preparation and bell pepper inoculation were carried out as previously outlined in the Bacterial Lawn Method Validation procedure. Inoculation of peppers was carried out by dipping the pepper in a sterile beaker containing the bacterial cocktail for 1 min. The peppers were then drip-dried for 1 h at room temperature. After peppers dried, peppers were chopped by first removing the stem area, followed by preparation of lengthwise latitudinal slices at a width of 1 cm. The pepper was chopped into squares with a surface area of 2 cm² (Large), 1 cm² (Medium), or 0.5 cm² (Small). After chopping, the knives were sampled using a swab, 1 swab per side of knife blade, totaling 2 swabs per knife (Table 5). Statistical analysis showed no significant difference between the large chop method and the medium chop method. There was also no significant difference between the small chop method and the medium chop method concentration of cells attached to knife blade.

Table 5- Chopping validation results.^a

Chopping Method^b	<i>Salmonella</i> (log₁₀ CFU/cm²)	<i>E. coli</i> O157:H7 (log₁₀ CFU/cm²)
Large	3.5 ± 0.5 ^a	3.6 ± 0.5 ^a
Medium	3.2 ± 0.4 ^{ab}	3.3 ± 0.5 ^{ab}
Small	2.9 ± 0.2 ^b	3.0 ± 0.5 ^b
Sampled Inoculated Pepper ^c	4.3 ± 0.3	4.1 ± 0.3
	<i>Salmonella</i> (log₁₀ CFU/ml)	<i>E. coli</i> O157:H7 (log₁₀ CFU/ml)
Inoculum	8.5 ± 0.0	8.4 ± 0.0

^aTable depicts means of triplicate replications with mean separation by Fisher's LSD; means within the same column with the same letter are not significantly different ($p \geq 0.05$).

^bPeppers were chopped large (2 cm²), medium (1 cm²) and small (0.5 cm²).

^cFollowing inoculation of the peppers, pepper samples consisting of three 10 cm² excisions/sample were taken in duplicate to determine bacterial concentration on the surface of the peppers.

Study 1: Antimicrobial Disinfection of Pepper Surfaces

A cocktail of rifampicin-resistant *Salmonella* and *E. coli* O157:H7 was dip inoculated on to waxed green bell peppers to a surface concentration of approximately $5.6 \pm 0.5 \log_{10}$ CFU/cm². Microbiological analysis of background microbiota on non-inoculated control peppers throughout Study 1 resulted in a mean APC of $3.7 \pm 0.6 \log_{10}$ CFU/cm², a mean LAB concentration of $2.2 \pm 1.0 \log_{10}$ CFU/cm², and a mean yeast and mold concentration of $2.8 \pm 0.3 \log_{10}$ CFU/cm². Duplicate samples consisting of 3 10-cm² pieces of inoculated pepper were excised from smooth tissue and immersed in 3% (v/v) H₂O₂ (pH 4.6 ± 0.2), 2.5% (v/v) acetic acid (pH 2.6 ± 0.0), 70% (v/v) EtOH (pH 7.0 ± 1.1), or SDW (pH 7.2 ± 0.4) for 0, 0.25, 0.5, 1, 2, 3, 4, or 5 min. For *Salmonella*, exposure to H₂O₂ achieved a reduction of $1.3 \pm 0.4 \log_{10}$ CFU/cm² after 5 min (Table 6)

and a reduction of $1.3 \pm 0.1 \log_{10}$ CFU/cm² for *E. coli* O157:H7 (Table 7). Following 1 min exposure to EtOH, *Salmonella* were reduced by $1.4 \pm 0.1 \log_{10}$ CFU/cm² (Table 6), and *E. coli* O157:H7 were reduced by $1.3 \pm 0.1 \log_{10}$ CFU/cm² (Table 7). Exposure of peppers to EtOH for >1 min did not result in additional reduction. Acetic acid exposure after 5 min resulted in a *Salmonella* reduction of $1.0 \pm 0.7 \log_{10}$ CFU/cm² (Table 6), but for *E. coli* O157:H7, exposure resulted in no significant reduction of pathogens compared to SDW at the various points (Table 7).

Table 6- Reduction of *Salmonella* serovars on bell peppers after various times of contact with different antimicrobials.^a

Dip Time (s)	Ethanol (70%)	Hydrogen Peroxide (3%)	Vinegar (2.5% Acetic Acid)	Sterile Distilled Water
0.25	0.9 ± 0.1^{bcdefg}	0.2 ± 0.5^{hij}	0.5 ± 0.2^{fghij}	0.0 ± 0.5^j
0.5	0.9 ± 0.3^{bcdefg}	0.8 ± 0.2^{bcdefgh}	0.4 ± 0.6^{hij}	0.1 ± 0.2^{ij}
1	1.4 ± 0.1^{abc}	0.8 ± 0.1^{abcdeghi}	1.0 ± 0.5^{abcdef}	0.2 ± 0.2^{ghij}
2	1.3 ± 0.1^{abcd}	0.5 ± 0.1^{efghij}	0.8 ± 0.6^{cdefghij}	0.7 ± 0.4^{defghij}
3	1.6 ± 0.2^{ab}	0.3 ± 0.9^{ghij}	0.8 ± 0.5^{bcdefghij}	0.5 ± 0.6^{efghij}
4	1.7 ± 0.7^a	0.7 ± 0.4^{cdefghij}	1.3 ± 0.5^{abcd}	0.2 ± 0.1^{hij}
5	1.5 ± 0.6^{ab}	1.3 ± 0.4^{abcde}	1.0 ± 0.7^{abcdef}	0.2 ± 0.2^{hij}

^aTable depicts mean log reductions of triplicate replications with duplicate samples (n=6) and mean separation by Fisher's LSD; means throughout the table with the same letter are not significantly different ($p \geq 0.05$).

Table 7- Reduction of *E. coli* O157:H7 on bell peppers after various times of contact with different antimicrobials.^a

Dip Time (s)	Ethanol (70%)	Hydrogen Peroxide (3%)	Vinegar (2.5% Acetic Acid)	Sterile Distilled Water
0.25	0.9 ± 0.2 ^{bcdef}	0.2 ± 0.4 ^{gh}	0.5 ± 0.1 ^{efgh}	0.2 ± 0.6 ^{fgh}
0.5	0.8 ± 0.2 ^{cdefgh}	1.0 ± 0.1 ^{abcde}	0.2 ± 0.5 ^h	0.2 ± 0.2 ^{fgh}
1	1.3 ± 0.0 ^{abcd}	0.9 ± 0.1 ^{bcdef}	0.7 ± 0.3 ^{cdefgh}	0.4 ± 0.2 ^{efgh}
2	1.4 ± 0.2 ^{abc}	0.7 ± 0.2 ^{cdefgh}	0.6 ± 0.6 ^{defgh}	0.8 ± 0.3 ^{cdefgh}
3	1.6 ± 0.1 ^a	0.5 ± 1.1 ^{efgh}	0.6 ± 0.6 ^{efgh}	0.8 ± 0.5 ^{cdefgh}
4	1.6 ± 0.7 ^{ab}	0.8 ± 0.2 ^{cdefg}	1.1 ± 0.5 ^{abcde}	0.5 ± 0.1 ^{efgh}
5	1.6 ± 0.1 ^a	1.4 ± 0.3 ^{abc}	0.7 ± 0.8 ^{cdefgh}	0.4 ± 0.2 ^{efgh}

^aTable depicts mean log reductions of triplicate replications with duplicate samples (n=6) and mean separation by Fisher's LSD; means throughout the table with the same letter are not significantly different (p≥0.05).

Study 2A: Efficacy of Decontamination of Knives

Inoculum preparation and inoculation of bell peppers were carried out as previously outlined in Study 1. Microbiological analysis of background microbiota on non-inoculated control peppers throughout Study 2A and 2B resulted in a mean APC $3.3 \pm 0.5 \log_{10}$ CFU/cm², a mean LAB concentration of $3.0 \pm 0.5 \log_{10}$ CFU/cm², and a mean yeast and mold concentration of $3.0 \pm 0.7 \log_{10}$ CFU/cm². After peppers dried for 60 min, peppers were chopped by first removing the stem area, followed by preparation of lengthwise latitudinal slices at a width of 1 cm. The pepper was chopped into squares with a surface area of approximately 1 cm². Knives were then treated with 1 of 5 cleaning methods: control (no treatment), a towel wipe and dry exposure, running hot water (60 °C) for 5 s, running hot water for 10 s and 1% (v/v) detergent solution and water followed by hot running water (60 °C) for 10 s. Statistical analysis determined that 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap cleaning methods

were not significantly different from each other in effectiveness of knife decontamination with mean reductions for *Salmonella* of $2.8 \pm 1.0 \log_{10}$ CFU/cm², $3.2 \pm 0.9 \log_{10}$ CFU/cm², and $3.7 \pm 0.3 \log_{10}$ CFU/cm² for 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap, respectively (Fig. 5). Mean reductions for *E. coli* O157:H7 of $3.3 \pm 0.6 \log_{10}$ CFU/cm², $3.3 \pm 0.7 \log_{10}$ CFU/cm², and $3.6 \pm 0.3 \log_{10}$ CFU/cm² were obtained following exposure of knives to 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap, respectively (Fig. 6). The towel wipe cleaning method was the least effective when compared to the other cleaning methods with a mean reduction of *Salmonella* of $2.1 \pm 0.9 \log_{10}$ CFU/cm² and mean reduction of *E. coli* O157:H7 of $1.8 \pm 1.0 \log_{10}$ CFU/cm².

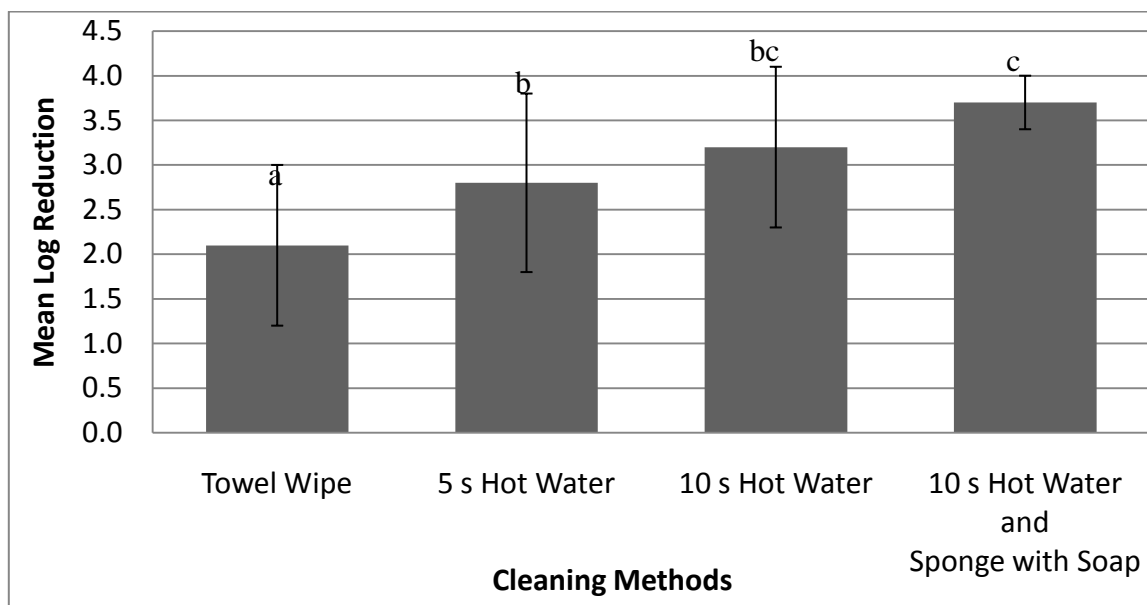


Figure 5- Efficacy of decontamination of knife through various cleaning methods for *Salmonella* serovars. Columns represent mean log reductions from triplicate replications with each replicate containing duplicate identical samples (n=6). Error bars indicate one standard deviation from the mean. Columns that have a common letter are not significantly different ($p \geq 0.05$).

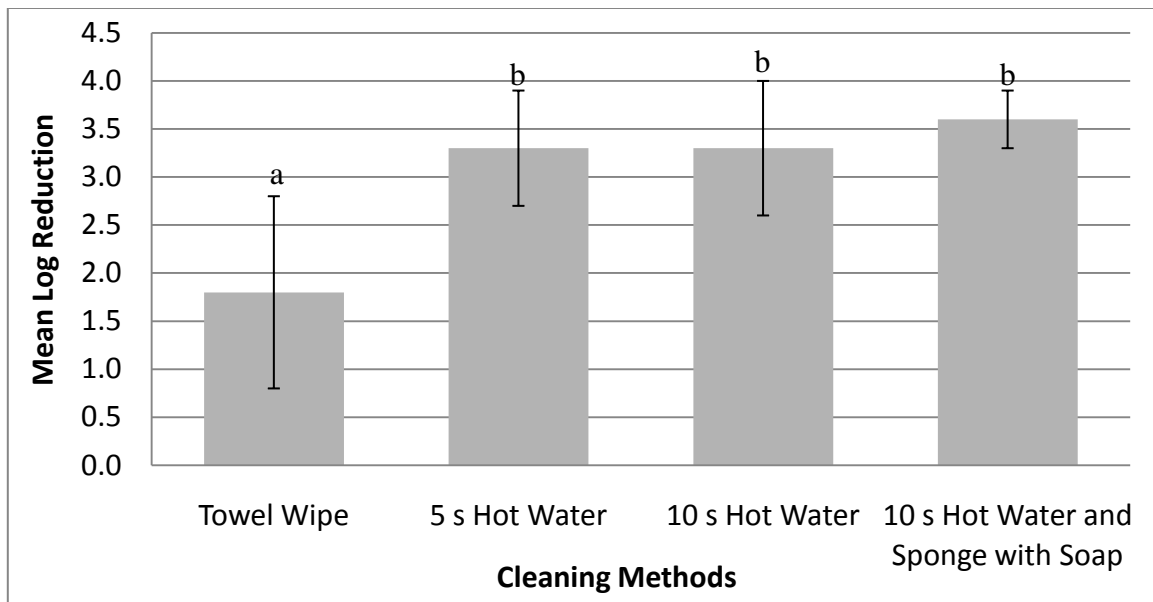


Figure 6- Efficacy of decontamination of knife through various cleaning methods for *E. coli* O157:H7. Columns represent mean log reductions from triplicate replications with each replicate containing duplicate identical samples (n=6). Error bars indicate one standard deviation from the mean. Columns that have a common letter are not significantly different ($p \geq 0.05$).

Study 2B: Transfer Testing

Inoculum preparation and inoculation of bell peppers was carried out as previously outlined. Sterile knives were used to chop peppers as previously outlined, and each knife was treated with 1 of the previously outlined treatments. The knives were then used to chop a cucumber that had been perforated using a sterile metal ruler to 2-mm thick slices and each sample consisted of 2 slices to total approximately 80 cm². Statistical analyses also showed there was not a significant difference between the mean log₁₀ CFU/cm² on the surface of the knife blade and the log CFU/cm² that was transferred onto the surface of the cucumber throughout Study 2B for both *Salmonella* (Fig. 7) and *E. coli* O157:H7 (Fig. 8). Mean *Salmonella* counts on the surfaces of knives

not treated (controls), towel wiped, 5 s hot water-treated, 10 s hot water-treated, and 10 s hot water plus soap-treated were $3.6 \pm 0.5 \log_{10} \text{CFU/cm}^2$, $1.4 \pm 0.5 \log_{10} \text{CFU/cm}^2$, $0.8 \pm 0.8 \log_{10} \text{CFU/cm}^2$, $0.7 \pm 1.0 \log_{10} \text{CFU/cm}^2$, and $0.0 \pm 0.0 \log_{10} \text{CFU/cm}^2$, respectively (Fig. 7). Transfer of *Salmonella* onto the surface of the cucumber slices resulted in mean counts of $3.9 \pm 0.8 \log_{10} \text{CFU/cm}^2$, $1.9 \pm 1.0 \log_{10} \text{CFU/cm}^2$, $1.1 \pm 1.3 \log_{10} \text{CFU/cm}^2$, $0.5 \pm 0.5 \log_{10} \text{CFU/cm}^2$, and $0.1 \pm 0.2 \log_{10} \text{CFU/cm}^2$ for controls, towel wiped, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap-exposed knives, respectively (Fig. 7). Mean *E. coli* O157:H7 counts on the surface of knife blades were $3.7 \pm 0.6 \log_{10} \text{CFU/cm}^2$, $1.1 \pm 0.6 \log_{10} \text{CFU/cm}^2$, $0.4 \pm 0.7 \log_{10} \text{CFU/cm}^2$, $0.5 \pm 0.8 \log_{10} \text{CFU/cm}^2$, and $0.0 \pm 0.0 \log_{10} \text{CFU/cm}^2$ for controls, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap, respectively (Fig. 8). Transfer of *E. coli* O157:H7 onto the surface of the cucumber slices resulted in mean counts of $3.6 \pm 0.8 \log_{10} \text{CFU/cm}^2$, $2.6 \pm 0.9 \log_{10} \text{CFU/cm}^2$, $0.3 \pm 0.5 \log_{10} \text{CFU/cm}^2$, $0.2 \pm 0.4 \log_{10} \text{CFU/cm}^2$, and $0.0 \pm 0.0 \log_{10} \text{CFU/cm}^2$ for controls, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap respectively (Fig. 8). Statistical analyses showed no difference between pathogen adherence to the sponge after wiping of the knife blade ($4.2 \pm 0.6 \log_{10} \text{CFU/cm}^2$ *Salmonella* and $4.1 \pm 0.6 \log_{10} \text{CFU/cm}^2$ *E. coli* O157:H7) when compared to pathogen adherence to the towel after wiping ($4.4 \pm 0.7 \log_{10} \text{CFU/cm}^2$ *Salmonella* and $4.3 \pm 0.7 \log_{10} \text{CFU/cm}^2$ *E. coli* O157:H7).

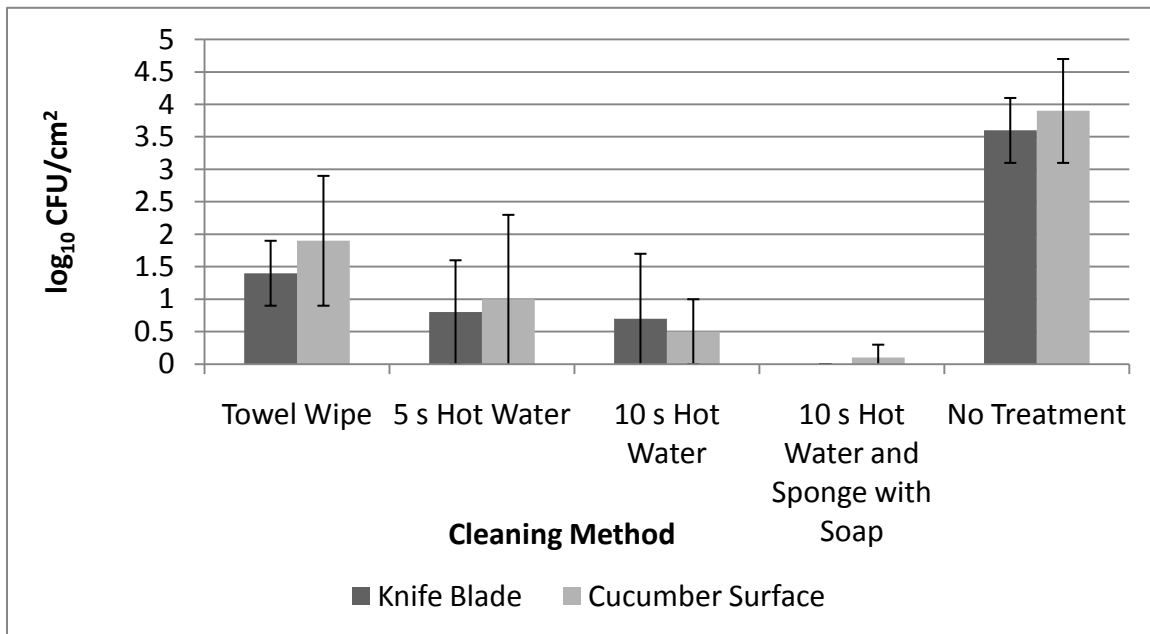


Figure 7- Transfer of *Salmonella* serovars on the surfaces of contaminated green bell peppers to non-treated salad cucumbers via contaminated knives after various cleaning methods. Columns represent mean survivors on knife blade and cucumber surface from triplicate replications with each replicate containing duplicate identical samples (n=6). Error bars indicate one standard deviation from the mean. Enrichment detected growth of *Salmonella* when counts were below the detection limits (1 CFU/cm²).

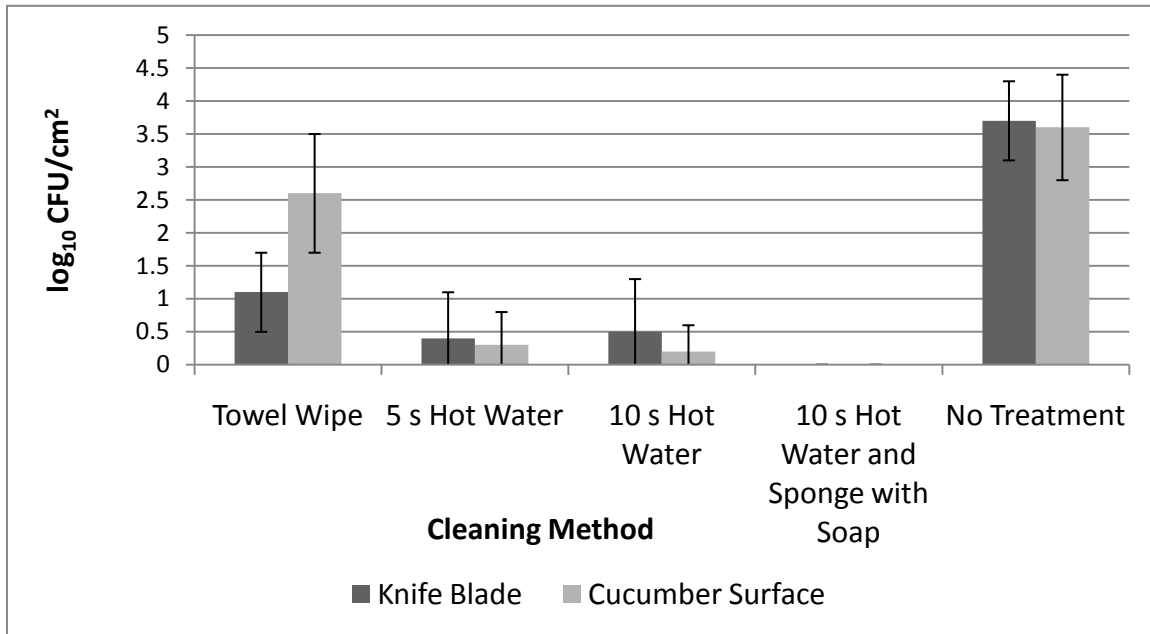


Figure 8- Transfer of *E. coli* O157:H7 on the surfaces of contaminated green bell peppers to non-treated salad cucumbers via contaminated knives after various cleaning methods. Columns represent mean survivors on knife blade and cucumber surface from triplicate replications with each replicate containing duplicate identical samples (n=6). Error bars indicate one standard deviation from the mean. Enrichment detected growth of *E. coli* O157:H7 when counts were below the detection limits (1 CFU/cm²).

CHAPTER VI

DISCUSSION

The preliminary studies determined no significant difference between the various dip times with respect to bacterial attachment to the surface of the bell pepper. The spot inoculation method showed to be effective in allowing adherence of *Salmonella* and *E. coli* O157:H7 to surfaces of the bell pepper with no significant difference in inoculums made with 0.1% Peptone and inoculums made with TSB. For Studies 1, 2A, and 2B the dip inoculation method (dip for 1 min) was chosen due to the overall process of inoculation being more time efficient than the spot inoculation method. The bacterial lawn method provided a higher initial bacterial concentration; thus, this method was used for the growth of *Salmonella* spp. and *E. coli* O157:H7 throughout the studies.

Acetic acid exposure resulted in no reduction of *E. coli* O157:H7 compared to SDW at the various time points up to 5 min. Antimicrobial activity of acetic acid does depend on the exposure time, temperature, concentration of acid, dissociation level, and/or pH, and the effectiveness of acetic acid increases as its concentration increase, pH decreases, temperature increases, and microbial load decreases (Naidu 2000). The tested concentration of acetic acid was 2.5%, and at this percentage the results support other previously reported experiments suggesting that a longer exposure time would be necessary for significant reduction. Entani and others (1998) determined that the time necessary for vinegar containing 2.5% acetic acid to inactivate *E. coli* O157:H7 was 150 min. Also, a study by Segun and Karapinar (2005) showed vinegar at a higher concentration of acetic acid (3.95%) could be used as a natural sanitizer at the household

level for the removal of *Salmonella* on spring onion, but this study showed that an exposure to vinegar after a 60 min treatment reduced *S. Typhimurium* 2.1 and 2.9 log₁₀ CFU/g. Another study compared the effectiveness of vinegar but on shredded carrots inoculated at 5.7 log₁₀ CFU/ml (Sengun and Karapinar 2004). The study showed vinegar containing a higher concentration of acetic acid (4.03%) reduced *S. Typhimurium* 1.9, 2.6, and 3.6 log₁₀ CFU/g after 15, 30, and 60 min of exposure with no significant statistical difference at 15 and 30 min of exposure (Sengun and Karapinar 2004). Thus, studies have shown that vinegar could be used effectively as a household antimicrobial but at longer exposure times and possibly at a higher concentration of acetic acid.

As with other antimicrobials, the speed and activity of the antimicrobial is influenced by various factors such as the concentration of the antimicrobial and the temperature (Davidson and Branen 1993). All the antimicrobials in the Study 1 were used at room temperature 22 °C. Hydrogen peroxide was used at a concentration of 3% and showed significant reduction of both pathogens at 5 min of exposure. Previous experiments in apples have shown its effectiveness at a 5% concentration and 3.0 log₁₀ CFU/g reduction of log₁₀ CFU/g of *E. coli* O157:H7 (Sapers and others 2000). Fresh-cut cantaloupe showed that both 2.5% and 5% concentrations yielded a reduction of *Salmonella* of 2.0 log₁₀ CFU/g, and the fresh-cut honeydew yielded a reduction of 1.3 log₁₀ CFU/g (Ukuku 2004).

Ethanol has been used as an antimicrobial since the first alcoholic fermentation was practiced to preserve fruit by denaturing proteins in the cytoplasm and is bactericidal at high concentrations (Davidson and Branen 1993). EtOH has also been

found to be effective in reducing pathogens on the surface of produce (Beier and others 2004), yet its efficacy compared to other household antimicrobials has not been fully explored. This study showed that EtOH at a concentration of 70% is effective in reducing pathogens when compared to SDW at times greater than 1 min. Therefore, when compared to vinegar (2.5% acetic acid) and H₂O₂ (3%) for this study EtOH was more effective as significant decontamination occurred at a shorter exposure time.

For studies 2A and 2B, preliminary studies showed that chopping the bell pepper to pieces of 1 cm² was not significantly different from a chopping size of 2 cm² and 0.5 cm² in bacterial attachment to the knife blade. Thus, the chopping size 1 cm² was used for studies 2A and 2B. Preliminary studies also showed that both a swab method of sampling the knife blade and the sponge method of sampling the knife blade were not significantly different for each other in sampling of the knife blade. For studies 2A and 2B, the swab method was used.

For study 2A, statistical analysis determined that 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap cleaning methods were not significantly different from each other in effectiveness of knife decontamination with mean reductions for *Salmonella* serovars of $2.8 \pm 1.0 \log_{10}$ CFU/cm², $3.2 \pm 0.9 \log_{10}$ CFU/cm², and $3.7 \pm 0.3 \log_{10}$ CFU/cm², respectively. Mean reductions for *E. coli* O157:H7 of $3.3 \pm 0.6 \log_{10}$ CFU/cm², $3.3 \pm 0.7 \log_{10}$ CFU/cm², and $3.6 \pm 0.3 \log_{10}$ CFU/cm² were obtained for 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap, respectively. The towel wipe cleaning method was the least effective when compared to the other cleaning methods with a mean reduction of *Salmonella* serovars of $2.1 \pm 0.9 \log_{10}$ CFU/cm² and a

reduction of $1.8 \pm 1.0 \log_{10}$ CFU/cm² for *E. coli* O157:H7. These results do agree with previous studies. Mattick and others (2003) showed that contaminated surfaces such as sponges, cloths, or plates can contaminate other surfaces or foods. Goulter and others (2008) determined the effect of combinations of time and temperature ranging from 1 s to 60 s and 60 °C to 82 °C on the disinfection of knives artificially contaminated with *E. coli*. The study showed that at all temperatures, increasing the length of immersion from 1 to 5 s gave a significant increase in bacterial reduction ($p < 0.01$), and for temperatures of 70 °C and above, increasing the immersion time from 5 to 10 s also significantly increased the bacterial reduction ($p < 0.01$) (Goulter and others 2008).

For study 2B, statistical analysis also showed there was not a difference between the mean \log_{10} CFU/cm² on the surface of the knife blade and the \log CFU/cm² that was transferred onto the surface of the cucumber throughout Study 2B for both *Salmonella* (Fig. 7) and *E. coli* O157:H7 (Fig. 8). Mean *Salmonella* counts on the surface of the knife blade were $3.6 \pm 0.5 \log_{10}$ CFU/cm², $1.4 \pm 0.5 \log_{10}$ CFU/cm², $0.8 \pm 0.8 \log_{10}$ CFU/cm², $0.7 \pm 1.0 \log_{10}$ CFU/cm², and $0.0 \pm 0.0 \log_{10}$ CFU/cm² for no treatment, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap respectively (Fig. 7). Transfer of *Salmonella* onto the surface of the cucumber slices resulted in mean counts of $3.9 \pm 0.8 \log_{10}$ CFU/cm², $1.9 \pm 1.0 \log_{10}$ CFU/cm², $1.1 \pm 1.3 \log_{10}$ CFU/cm², $0.5 \pm 0.5 \log_{10}$ CFU/cm², and $0.1 \pm 0.2 \log_{10}$ CFU/cm² for no treatment, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap respectively (Fig. 7). Mean *E. coli* O157:H7 counts on the surface of the knife blade were $3.7 \pm 0.6 \log_{10}$ CFU/cm², $1.1 \pm 0.6 \log_{10}$ CFU/cm², $0.4 \pm 0.7 \log_{10}$ CFU/cm², $0.5 \pm 0.8 \log_{10}$ CFU/cm²,

and $0.0 \pm 0.0 \log_{10} \text{CFU/cm}^2$ for no treatment, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap respectively (Fig. 8). Transfer of *E. coli* O157:H7 onto the surface of the cucumber slices resulted in mean counts of $3.6 \pm 0.8 \log_{10} \text{CFU/cm}^2$, $2.6 \pm 0.9 \log_{10} \text{CFU/cm}^2$, $0.3 \pm 0.5 \log_{10} \text{CFU/cm}^2$, $0.2 \pm 0.4 \log_{10} \text{CFU/cm}^2$, and $0.0 \pm 0.0 \log_{10} \text{CFU/cm}^2$ for no treatment, towel wipe, 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap respectively (Fig. 8). Statistical analyses showed no significant difference between how much adhered to the sponge after wiping of the knife blade (*Salmonella* for $4.2 \pm 0.6 \log_{10} \text{CFU/cm}^2$ and $4.1 \pm 0.6 \log_{10} \text{CFU/cm}^2$ for *E. coli* O157:H7) when compared to how much adhered to the towel after wiping (*Salmonella* for $4.4 \pm 0.7 \log_{10} \text{CFU/cm}^2$ and $4.3 \pm 0.7 \log_{10} \text{CFU/cm}^2$ for *E. coli* O157:H7). These results suggest that viable organisms remained on the knife blade after each cleaning method have the potential to contaminate another food or food contact surface. Kusumaningrum and others (2003) also determined that pathogens remain viable on dry stainless steel surfaces and present contamination hazard for considerable periods of time, depending on the contamination levels and the type of pathogen. Mattick and others (2003) examined the risk of bacterial transfer onto sterile dishes and sponges via contaminated water, kitchen surfaces wiped with a contaminated sponge, items placed in direct contact with a contaminated kitchen surface, food placed on a contaminated dish or dishes from contaminated food, showing that viable organisms remaining on surfaces can contaminate other surfaces that come in direct contact.

CHAPTER VII

CONCLUSIONS

Study 1 determined the efficacy of the consumer-available antimicrobials hydrogen peroxide (3%), vinegar (2.5% acetic acid), and EtOH (70%) for the disinfection of *Salmonella* serovars and *Escherichia coli* O157:H7 from the surfaces of green bell peppers. The studied showed that H₂O₂ exposure for 5 min resulted in significant reductions for both *Salmonella* serovars and *E. coli* O157:H7, respectively. Exposure of peppers to EtOH for 1 min resulted in significant reductions of both pathogens from pepper surfaces, but that EtOH for >1 min did not result in additional reduction. Acetic acid exposure resulted in no reduction of pathogens compared to SDW. Findings suggest EtOH and H₂O₂ may be effective consumer-deployable antimicrobials for surface decontamination of smooth produce.

Study 2A determined the efficacy of consumer cleaning methods for decontamination of knives used to chop green bell peppers contaminated with *Salmonella* serovars and *E. coli* O157:H7. Statistical analysis determined that 5 s hot water, 10 s hot water, and 10 s hot water with sponge and soap cleaning methods were not significantly different from each other in effectiveness of knife decontamination. The method of using the towel to wipe the knife blade was the least effective cleaning method for the knife blade. Findings suggest that the rinsing with hot water is useful in knife decontamination in the home kitchen.

Study 2B assessed the potential for transfer of *Salmonella* serovars and *E. coli* O157:H7 on the surfaces of artificially contaminated green bell peppers to a non-treated

produce item (salad cucumbers) via contaminated knives. This study confirmed the findings from Study 2A in regards to the efficacy of the cleaning methods, and showed that viable organisms remaining on the surface of a knife blade after cleaning have the potential to contaminate another food or contact surface, in this case another produce item (salad cucumber).

Further research is needed on effect of temperature on the efficacy of these antimicrobials at the various time points as well as the impact on antimicrobial efficacy of longer exposure times. Further research of antimicrobial exposure on produce sensorial characteristics is also advised in order to determine how various antimicrobial exposure times will affect the quality and sensorial characteristics of the produce commodity.

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