CHIH-YING LU

Application of Physical and Chemical Means to Kill Foodborne Pathogens on Alfalfa Seeds

(Under the Direction of. MARK A. HARRISON)

Most foodborne illness outbreaks which alfalfa sprouts have been implicated have been associated with seeds contaminated with human pathogenic bacteria. The efficacy of ultrasound treatment with combinations of heat and chemical antimicrobial solutions was investigated. Significant ($P \leq 0.05$) reductions of over 4.33 log CFU/g of *Salmonella* populations were reached with ultrasound in combination with 1% $Ca(OH)_2$ at 55°C for 5 min, 1% $Ca(OH)_2$ plus 1% Tween 80 at 23 or 55°C for 2 or 5 min, and 8% H_2O_2 at 23°C for 5 min, 55°C for either 2 min without inhibiting the germination percentage of seeds. However, none of these treatments can completely eliminated the populations of *Salmonella* on seeds. Treatment of seeds with with 331 ppm Tsunami® 200 and 1200 ppm Sanova® were less effective in killing *Salmonella* on alfalfa seeds. Dielectric heating alone also caused significant reductions of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on alfalfa seeds without inhibiting the germination rate. When the seed moisture content was *ca.* 6.5%, dielectric heating reduced *E. coli O17:H7* populations by 1.14 log at 89°C for 20 sec; *Salmonella* populations by 1.06 log at 89°C for 20 sec; and *L. monocytogenes* by 0.89 log at 82°C for 12 sec. The overall appearance and color of sprouted seeds after treatment were not significantly different with untreated sprouted seeds.

INDEX WORDS: Alfalfa sprouts, Dielectric heating, Ultrasound, Heat, Sanitizing, Chemicals, *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella*.

APPLICATION OF DIELECTRIC HEATING AND ULTRASOUND IN COMBINATION WITH HEAT AND CHEMICAL SOLUTIONS TO KILL HUMAN PATHOGENIC BACTERIA ON ALFALFA SEEDS

by

CHIH-YING LU

B.S., National Taiwan Ocean University, Taiwan, 1999

A Thesis Submitted to the Graduate Faculty

of The University of Georgia in Partial Fulfillment

of the

Requirement for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2001

APPLICATION OF PHYSICAL AND CHEMICAL MEANS TO KILL FOODBORNE PATHOGENS ON ALFALFA SEEDS

By

CHIH-YING LU

Approved:

Major Professor: Mark A. Harrison

Committee: Larry R. Beuchat
Stuart O. Nelson

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
December 2001

Dedicated to the memory of

My Mom

ACKNOWLEDGEMENTS

I am deeply grateful to my major professor, Dr. Harrison, for inspiring guidance, substantial suggestions and understanding throughout this research.

I also want to take this opportunity to express my sincere gratitude to other committee members, Dr. Beuchat and Dr. Nelson, for their valuable comments and suggestions in thesis and the consistent support throughout my study.

Thanks to RuthAnn Marrow for the excellent technical assistance.

A special thanks to my boyfriend, Xinyu, for his love, support and encouragement.

Finally, I would like to dedicate this thesis to my mom, dad and brother who have been loving me and supporting me. I would not be where I am today without them.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Sprouts are a 250 million dollar market in the United States, with approximately 10 percent of Americans eating sprouts regularly (Kurtzweil, 1999). Sprouts are usually consumed raw and are common ingredients of vegetable salads. Sprouts contain carbohydrates, proteins, minerals and vitamins. The high nutritional value of sprouts makes them a valuable food for all consumers (Feng, 1997). However, the consumption of seed sprouts has been linked to many foodborne illness outbreaks in the United States and other countries.

Foodborne Illness Associated with Sprouts

Many microbiological investigations have shown the presence of a variety of foodborne pathogens in sprouts. Human pathogens associated with sprouted seeds and beans include *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* (Beuchat, 1996; Jackson 1998). *Aeromonas hydrophilia* and *Klebsiella pneumoniae* were also isolated from seeds and sprouts (Park and Sanders, 1990; Callister, 1987). From 1995 to 1999, more than ten outbreaks associated with contaminated sprouts have occurred in the United States. Other countries, including the United Kingdom, Sweden, Finland, Japan, Denmark and Canada also reported many sprout-associated foodborne illness outbreaks (NACMCF, 1999) (Tables 1.1, 1.2 lists the reported sprout outbreaks). Outbreak investigations have shown that microorganisms found on sprouts most likely originated from the seeds (NACMCF, 1999). In an outbreak of mung bean sprouts, *S.* Saint-Paul was isolated from the seeds used by the producer (O'Mahony et al., 1990). Two outbreaks occurred in Oregon and British Columbia in 1995 and 1996 and were caused by alfalfa seeds contaminated with *Salmonella* enteria Serotype Newport. However, unsanitary sprouting practices may also have contributed to the

Table 1.1 Reported U.S. outbreaks of foodborne illness due to sprout consumption, 1973-1999[a]

Year	Pathogen	No. of cases	Location	Type of Sprout	Likely Source of Contamination	Reference
1973	*Bacillus cereus*	4	Texas	Soy, Cress, Mustard	Seed	Portnoy et al, 1976
1995	*S.* Stanley	242	17 States	Alfalfa	Seed	Mahon et al, 1997
1995-96	*S.* Newport	>133	7 States	Alfalfa	Seed	Van Beneden et al, 1999
1996	*S.* Montevideo/ Meleagridis	>500	California	Alfalfa	Sprouter/Seed	Farrar and Mohle-Boetani, 1999
1997	*S.* Infantis/Anatum	90	Kansas, Missouri	Alfalfa	Seed	Slutsker, 1998
1997	*E. coli* O157:H7	108	Michigan, Virginia	Alfalfa	Seed	CDC, 1997
1997-98	*S.* Senftenberg	60	California, Nevada	Clover/Alfalfa	Sprouter/Seed	Farrar and Mohle-Boetani, 1999
1998	*E. coli* O157:NM	8	California	Clover/Alfalfa	Seed	Farrar and Mohle-Boetani, 1999
1998	*S.* Havana/Cubana	18	California	Alfalfa	Seed	Farrar and Mohle-Boetani, 1999
1999	*S.* Mbandaka	75	Oregon, Washington	Alfalfa	Seed	Keene, 1999

[a]Adapted with modification from NACMCF (1999)

Table 1.2 Reported international outbreaks of foodborne illness due to sprout consumption, 1988-1997

Year	Pathogen	No. of cases	Location	Type of Sprout	Likely Source of Contamination	Reference
1988	*S.* Saint-Paul	143	United Kingdom	Mung Bean	Seed	O' Mahnony et al, 1990
1989	*S.* Gold-Coast	31	United Kingdom	Cress	Unknown	Joce et al, 1990
1994	*S.* Bovismorbificans	492	Sweden, Finland	Alfalfa	Seed	Ponka et al, 1995 Puohiniemi et al, 1997
1995	*S.* Stanley	114	Finland	Alfalfa	Seed	Kontiainen et al, 1996 Mahon et al, 1997
1995	*S.* Newport	?	Denmark, Canada	Alfalfa	Seed	Oregon Health Division, 1995 Aabo and Baggesen, 1997
1996	*E. coli* O157:H7	>6,000	Japan	Radish	Unknown	Nat'l Inst. Infect. Dis. and Infect. Dis. Ctrl Div., Ministry of Health and Welfare of Japan, 1997
1997	*S.* Meleagridis	78	Canada	Alfalfa	Seed	Buck et al, 1998
1997	*E. coli* O157:H7	126	Japan	Radish	Unknown	Gutierrez, 1997

[a]Adapted with modification from NACMCF (1999)

contamination of sprouts based on the *S*. Montevideo/*S*. Meleagridis outbreak in 1996 (NACMCF, 1999).

***Sprout-associated outbreaks due to E. coli* O157:H7**

Since *E. coli* O157:H7 was identified as a pathogen in 1982, it has been the cause of a series of foodborne illness outbreaks in the United States. The largest outbreak of *E. coli* O157:H7 in the U.S. was associated with consumption of ground beef (Doyle et al, 1997). Outbreaks also have been linked to unpasteurized cider (Besser et al, 1993; Steele, 1982) cantaloupe, watermelon, alfalfa, radish sprouts (Gutierrez, 1997) and leaf lettuce (CDC, 1995). In 1997, an outbreak of 108 cases of *E. coli* O157:H7 in Michigan and Virginia were independently associated with consuming alfalfa sprouts grown from the same seed lot. The outbreak strains in Michigan and Virginia were found indistinguishable through epidemiological method (CDC, 1997). In 1996 in Japan, white radish sprouts were implicated in the world's largest outbreak of *E. coli* O157:H7 in which about 6,000 cases occurred (Gutierrez, 1997). In 1997, a small outbreak again occurred with 126 cases in Japan, in which an identical strain of *E. coli* O157:H7 was isolated from patients and from radish sprouts obtained from a patient's refrigerator (Gutierrez, 1997). In 1998, a nonmotile strain of *E. coli* O157 caused confirmed illness cases in California and Nevada associated with eating alfalfa and clover sprouts produced by the same producer implicated in a *S*. Senftenberg outbreak (NACMCF, 1999).

E. coli O157:H7 *and Its Disease Characteristics*

E. coli O157:H7 is gram-negative, nonsporeforming rods. It grows well in broth media within the temperature range of 30 to 42^{o}C, but at temperatures above 44-45^{o}C it grows poorly (Doyle and Schoeni, 1984). *E. coli* O157:H7 is very tolerant to acid environments and can survive in apple cider (pH 3.6-4.0) (Zhao et al, 1993). The heat

resistance of stationary phase *E. coli* O157:H7 in apple juice, orange juice and white grape juice was D_{60° = 0.8 min, D_{60° = 1.1 min and D_{60° = 0.7 min, respectively (Mazzotta, 2001b). Hot water immersion treatments of whole apples at 80 and 95°C for 15 sec resulted in a 5 log reduction of *E. coli* O157:H7 on apple surfaces (Fleischman et al, 2000).

E. coli O157:H7 infections can cause severe abdominal cramps, short-lived fever, and non-bloody diarrhea to life threatening conditions such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). Sometimes the infections are asymptomatic (Doyle and Cliver, 1990). Symptoms of hemorrhagic colitis include abdominal pain usually followed by bloody diarrhea. Hemolytic uremic syndrome is a leading cause of renal failure in children (Doyle, 1991) and is characterized by a triad of renal insufficiency, microangiopathic hemolytic anemia, and thrombocytopenia. TTP mainly affects adults and has similar characteristics as HUS but involves brain damage (Doyle and Cliver, 1990).

Sprout-associated outbreaks due to Salmonella

The initial source of *Salmonella* is the intestinal tracts of animals. Humans can acquire this bacterium from contaminated foods such as poultry, eggs, dairy products, or water. However, salmonellae have been isolated from several types of fresh vegetables, including lettuce, fennel, numerous salad vegetables, and tomatoes. In 1988, beansprouts, imported mainly from Australia and Thailand, were implicated in a foodborne illness outbreak of 143 cases due to *S.* Saint-Paul in the United Kingdom (O'Mahony et al., 1990), and a study revealed beansprout samples analyzed in Thailand carried several serotypes of *Salmonella* (Jerngklinchan and Sartanu, 1993). Between March and June of 1995, there was a significant increase in the number of

cases of *S.* Stanley in Arizona, Michigan and Finland (Mahon et al., 1997). An epidemiological study revealed that alfalfa sprouts grown from contaminated seeds were the source (Feng, 1997; Mahon et al., 1997). In 1996, an outbreak of about 450 cases associated with consumption of alfalfa sprouts contaminated with *S.* Montevideo and *S.* Meleagridis occurred in California because of an unsanitary sprouting process. At the farm where the alfalfa seeds were grown, alfalfa was fertilized with chicken manure, and animal manure was found next to the alfalfa fields. Sprouts were implicated in a 1997outbreak in Kansas and Missouri due to *S.* Infantis and *S.* Anatum. The sprout types included alfalfa, radish, and snow pea and were produced at a single facility. The seed was locally grown and came from many surrounding farms (Pezzino et al., 1998).

Salmonella and Its Disease Characteristics

Salmonella is a gram-negative, motile, non-sporeforming rod bacterium, which can cause salmonellosis. Growth temperatures for *Salmonella* range from 5-45^{o}C. Growth is very slow at the extreme temperatures and more rapid in the optimal range of 35-37^{o}C (Doyle and Cliver, 1990). S*almonella* can grow at pH values ranging from 4.5 to 9.5, with an optimum pH for growth of 6.5 to 7.5 (Doyle, 1990). The heat resistance of stationary phase *Salmonella* in apple juice, orange juice and white grape juice was $D_{60^{\circ}C}$ = 0.28 min, $D_{60^{\circ}C}$ = 0.21 min and $D_{60^{\circ}C}$ = 0.44 min, respectively (Mazzotta, 2001b).

Human *Salmonella* infections are usually divided into several clinical conditions, which include enteric or typhoid fever, uncomplicated enterocolitis, and systemic infections with nontyphoidal *Salmonella*. Gastroenteritis with diarrhea develop within 48 hours after indigestion and are accompanied by fever, cramping, nausea, vomiting, chills. *S.* Typhi usually causes enteric fever, with an incubation period of 7 to 28 days.

Symptoms include prolonged fever, abdominal pain, malaise, sore throat, and anorexia (D'Aoust, 1997).

Occurrence of Listeria monocytogenes on sprouts/sprout seeds

The major safety concern with nonsterile, ready-to-eat products is the possible increase in the population of psychrotrophic pathogens, such as *L. monocytogenes*, in the absence of sensory defects. *L. monocytogenes* has been isolated from a wide variety of raw or processed vegetables and several cases of listeriosis have been linked to the consumption of vegetable products (Nguyen-The and Carlin, 1994). *L. monocytogenes* is associated with soil, plant, animal products, and food processing environments. Heisick et al. (1989) using FDA procedures determined the incidence of various *Listeria* spp., including *L. monocytogenes*, in various raw unwashed samples. They found that root crops such as potatoes and radishes more frequently carry viable listeriae than other vegetables because of their close association with soil. Because it is widespread in the environment, there are various ways for *L. monocytogenes* to contaminate either seeds or sprouts. *L. monocytogenes* can grow at refrigeration temperatures on a variety of produce, including sprouts (NACMCF, 1999; Lovett, 1989). This pathogen has been found in commercially produced sprouted seeds, but no cases of human listeriosis have been associated with those sprouts (NACMCF, 1999).

Listeria monocytogenes and Its Disease Characteristics

L. monocytogenes is a small (1.0-2.0 μ × 0.5 μ), gram-positive, facultatively anaerobic, rod-shaped bacterium, which can cause listeriosis (Gray and Killinger, 1966). *L. monocytogenes* is psychrotrophic. It grows best at 30-37^{o}C, but it thrives at refrigeration temperature. The growth of *L. monocytogenes* on asparagus, broccoli, and cauliflower stored at 4^{o}C, lettuce at 5^{o}C and chicory endive at 6.5^{o}C has been

reported (Beuchat, 1996). *L. monocytogenes* grows best in a neutral to alkaline medium (pH range 6-8) but will also grow over the pH range 4.1-9.6 (Jay, 1992). Among the nonsporeforming bacteria, *L. monocytogenes* is relatively more heat resistant. The heat resistance of *L. monocytogenes* was higher in peas (D_{60} = 1 min) and mushrooms (D_{60} = 0.7 min) than in other vegetables such as onions (D_{60} = 0.2 min), and broccoli (D_{60} = 0.6 min) (Mazzotta, 2001a).

Listeriosis, a disease that almost often affects immunocompromised people, is clinically defined when the organism is isolated from blood, cerebrospinal fluid, or an otherwise normally sterile site (e.g., placenta, fetus). Listeriosis infections in pregnant women may result in spontaneous abortion or stillbirth. In adults, listeriosis is characterized by the onset of severe symptoms including meningitis, septicemia, primary bacteremia, endocarditis, nonmeningitic central nervous system infection, and flu-like illness (Marth and Bahk, 1990).

Seed contamination

Contaminated seeds are the likely sources in most sprout-associated foodborne illness outbreaks (Puohiniemi et al., 1997; CDC, 1997; Mahon et al., 1997). Since seeds are raw agricultural products, they could be contaminated by a variety of potential sources in the field including contaminated agricultural water, use of inadequately managed manure as a fertilizer, location of fields assessed by rodents, birds and other wild animals, and inadequate worker hygiene (NACMCF, 1999). Pathogenic bacteria are usually carried on the surface and within the seed (Neergaard 1977).

Sprouts follow a complex path from farm to fork that includes planting, growing, harvesting and cleaning, followed by storage and transportation of the finished product. Microbial contamination can occur at any of these points in production and

distribution (Taormina et al., 1999). Sprouts have higher risk than other fresh produce because of the bacterial proliferation during the sprouting process. The high moisture environment, nutrients liberated by the seeds, decrease of trypsin inhibitor and warm temperatures support the growth of pathogenic bacteria (Hara-Kudo 1997; Patterson, 1980). Portony et al. (1976) reported that *B. cereus* populations in seeds had increased to 10^4 to 10^7 CFU/g in sprouts. Andrew et al. (1982) revealed that the population of *Salmonella* increased dramatically and reached hazardous levels during sprouting of mung bean and alfalfa sprouts. Jaquette et al. (1996) found that *Salmonella* populations increased about 3 logs CFU/g after sprouting from alfalfa seeds. Pathogen populations can exceed 10^7 CFU/g without adverse effect on the sprout appearance. Hara-Kudo (1997) investigated the increase of *E. coli* O157:H7 during growth of radish sprouts and found that *E. coli* O157:H7 populations proliferated 10^3 to 10^5 fold at the early stage of germination and plant growth.

Seed decontamination

Sanitizing is generally more effective in reducing microbial contamination of seeds than on seed sprouts due to lower levels of microorganisms and organic matter present on seeds than on sprouts. In addition, the infiltration of bacteria into sprout tissue during sprouting makes them physically more inaccessible to sanitizers (Hara-Kudo et al., 1997; Itoh et al., 1998; Caetano-Anolles et al., 1990).

Chemical treatment of seeds and sprouts

Numerous studies have been done to determine the effectiveness of chemicals in killing the usual microorganisms and pathogenic bacteria on seeds and sprouts.

Chlorine

Chlorine is used as a disinfectant in wash, spray and flume water in the raw fruit and vegetable industry. Inhibitory or lethal activity depends on the amount of free

available chlorine (as hypochlorous acid) that comes in contact with microbial cells. Chlorine rapidly loses activity on contact with organic matter or exposure to air, light or metals.

Piernas and Guiraud (1997) investigated several methods of disinfection of rice seeds. Treatment with 1,000 ppm sodium hypochlorite for 20 min at room temperature resulted 2 to 3 log reductions in aerobic plate counts from seeds without inhibiting the germination. Benzalkonium chloride (1%) eliminated 2.5 to 3 logs of APC in 10 min on rice seeds without adversely affecting germination; however, the residues could remain on the seeds (Piernas and Guiraud, 1997). Soaking of mung bean sprouts for 30 min or 60 min in 0.5% sodium hypochlorite resulted in a 2 log decrease in counts (Piernas and Guiraud, 1997).

Dipping Brussels sprouts into a 200 ppm chlorine solution for 10 sec decreased the populations of *Listeria moncytogenes* (10^6 CFU/g of initial population) by about 100 fold (Brackett, 1987). Jaquette et al. (1996) investigated the efficacy of chlorine in killing *S.* Stanley inoculated alfalfa seeds. They revealed that 100-1010 μg/ml chlorine treatment for 5 or 10 min resulted a significant reduction (about 1.5 log) of *S.* Stanley. However, chlorine at 1,010-μg/ml failed to cause a further reduction. This may be due to the infiltration of *S.* Stanley into seeds through cracks and crevices, and the rapid converting of active chlorine to inactive form on contact with high levels of organic material (such as alfalfa seeds). Treatment of seeds in 2040 μg/ml chlorine solution reduced 10^1-10^2 CFU/g of *S.* Stanley to an undetectable level (< 1 CFU/g). The sensory quality of sprouts produced from seeds receiving this treatment is not adversely affected.

Taormina and Beuchat (1999) determined the efficacy of various chemicals in killing *E. coli* O157:H7 on alfalfa seeds. Significant reduction in the population

(nearly 1 log) of *E. coli* O157:H7 was noted on alfalfa seeds after treatment with 500 and 1,000 ppm of active chlorine for 3 min and with ≥ 2,000 ppm of Ca $(OCl)_2$ for 3 or 10 min. Treatment with 20,000 ppm of active chlorine (Ca $(OCl)_2$) failed to eliminated 2.68 log CFU/g. Treatment with 500 ppm acidified ClO_2 significantly reduced the populations of *E. coli* O157:H7 from 2.7 to < 0.5 CFU/g. Treatment with either 20,000 ppm of chlorine or Fit® (a liquid prototype produce wash product) on alfalfa seeds resulted in significant reductions (ranging from 4-4.7 log) in populations of *Salmonella* and *E. coli* O157:H7 compared with 200 ppm chlorine (Beuchat et al., 2001). Lang (2000) found that treatment with 20,000 ppm active chlorine of *E. coli* O157:H7 inoculated alfalfa seeds for 15 min at 25°C was the most lethal treatment which resulted in a 6.9 log reduction compared to treatment with an organic acid/2,000 ppm chlorine mixtures or organic acid treatment alone. The regrowth of *E. coli* O157:H7 on treated alfalfa seeds occurred during sprouting.

H_2O_2

Hydrogen peroxide cytotoxicity is due to its capacity to generate powerful oxidants such as hydroxyl radicals that can initiate lipid oxidation chain reactions and damage to nucleic acids, proteins and lipids in bacterial cells (Juven and Pierson, 1996). Treatment with 1% hydrogen peroxide for 10 min at room temperature resulted in a 2 log reduction in aerobic plate counts on rice seeds without inhibiting the germination (Piernas and Guiraud, 1997). Treatment with ≥ 1% H_2O_2 significantly reduced *E. coli* O157:H7 on alfalfa seeds with an initial count of 3.21 log to an undetectable level (Taormina and Beuchat, 1999). More than a 3-log reduction in the population of *Salmonella* was obtained after treatment of alfalfa seeds with 1,800-2,000 µg/ml hypochlorite or 10% hydrogen peroxide for 10 min without significantly

inhibiting the germination; however, the presence of *Salmonella* was revealed by subsequent enrichment (Beuchat, 1997). Treatment with 8% H_2O_2 on alfalfa seeds caused reduction in the population of *Salmonella* by 3.2 log CFU/g (Weissinger and Beuchat, 2000).

Organic acids

The mode action of organic acids inhibiting microbial growth is due to direct pH reduction, depression of the internal pH of microbial cells by ionization of the undissociated acid molecule, or disruption of substrate transport by alteration of cell membrane permeability. Peroxyacetic acid has been used as a sanitizer for food processing equipment and shown effectiveness against biofilm (Beuchat, 1998). Treatment of ready-to-eat salads with peroxyacetic acid with 90 ppm has been shown to reduce total counts and fecal coliforms by about 100 fold (Beuchat, 1998). Application of peroxyacetic/octanoic acid mixtures showed a significant reduction in the populations of fecal coliform bacteria and fungi on the fresh-cut-vegetables and its processing water, respectively (Hilgren and Salverda, 2000).

Taormina and Beuchat (1999) also obtained significant reductions (ranging from 1.5-2.1 log) in populations of *E. coli* O157:H7 after treatment with 40 ppm of Tsunami™ and Vortexx™, and 1% Vegi-Clean™ for 3 or 10 min. Lang et al. (2000) investigated the efficacy of using organic acid plus hypochlorite treatment for 15 min at 25^{o}C for eliminating *E. coli* O157:H7 from alfalfa seeds. They found that treatment with 5% lactic acid for 10 min at 42^{o}C followed by treating with 2,000 ppm active chlorine for 15 min at 25^{o}C resulted in a reduction of 4.1 log CFU/g. Other treatments, which used organic acid/2,000 ppm chlorine combinations or organic acid alone are less effective. None of the treatments could prevent the regrowth of *E. coli* O157:H7 on treated alfalfa seeds during sprouting.

Weissinger and Beuchat (2000) investigated aqueous chemical treatments for eliminating *Salmonella* on alfalfa seeds. Treatment of alfalfa seeds with 20,000 ppm free chlorine Ca $(OCl)_2$ for 10 min eliminated 2 log CFU/g. Treatment with 2,000 ppm $NaClO_2$ and 1,060 ppm Tsunami™ or Vortex ™ was less effective in reducing the population of *Salmonella* on alfalfa seeds. Presoaking seeds in water or various chemical solutions (such as Tween 80, EDTA or Tween 80 plus EDTA) did not substantially influence the efficacy of the subsequent chemical treatments.

Calcinated calcium and calcium hydroxide

The antibacterial capacity of calcinated calcium or calcium hydroxide is attributed to its ionic disassociation and is directly related to its elevated pH which can cause bacterial enzymatic inactivation. Bari (1999) investigated the inhibitory effect of calcinated calcium on the growth of *E. coli* O157:H7 during fresh radish sprout production. This study revealed that the use of 0.4% calcinated calcium completely inhibited the microorganism (3.0-3.2 log CFU/ml reduction). Weissinger and Beuchat (2000) found that treatment of alfalfa seeds with 1% Ca $(OH)_2$ and 1% calcinated calcium caused reduction in populations of *Salmonella* by 2.8 and 2.9 log CFU/g, respectively. Treatment with 1% Ca $(OH)_2$ plus 1% Tween 80 was more effective in eliminating *Salmonella* than 1% Ca $(OH)_2$ treatment alone (Weissinger and Beuchat, 2000).

Heat treatment

Washing sprouts with water decreased numbers of *B. cereus*, *E. coli* or *Salmonella* by no more than 1 log (Harmon et al., 1987; NACMCF, 1999). Piernas and Guiraud (1997) found that aerobic plate count from rice seeds was reduced by approximately 3 logs and the fungal population was decreased by 1 log after treatment with hot water at 60^{o}C for 5 min. Treatment of seeds in water at 54^{o}C for 5 or 10 min

resulted 1.6 log reduction of *S.* Stanley. However, treatment at >54^{o}C significantly affected viability of the seeds (Jaquette et al., 1996). They also revealed that up to 5 log reduction of APC was obtained by soaking seeds for 5 min in 1,000 ppm sodium hypochlorite solution at 60^{o}C (Piernas and Guiraud, 1997). *Listeria innocua* population was reduced by about 2.5 logs after treatment of rice seeds with hypochlorite at 60^{o}C for 5 min (Piernas and Guiraud, 1998). Treatment of mung bean seeds with 242 µl gaseous acetic acid per liter of air for 12 h at 45^{o}C reduced the populations of *S.* Typhimurium, *E. coli* O157:H7, and *Listeria* by approximately 5, 6, and 4 logs, respectively. Neither *S.* Typhimurium nor *E. coli* O157:H7 were detected by enrichment of the seeds (Delaquiset et al, 1999).

Ultrasound

The use of ultrasound techniques for the decontamination of foods has been demonstrated previously (Curra and Tamsma, 1960). The bactericidal effect of ultrasound is generally attributed to intracellular cavitation (Hughes and Nyborg, 1962). Ultrasound processing causes micromechanical shocks by making and breaking microscopic bubbles. These shocks disrupt cellular structural and functional components leading to cell lysis (FDA, 2000; Vollmer, 1998).

Lee et al. (1989) found that ultrasonic treatment (10 min) of *Salmonella* in peptone water resulted in a 4 log population reduction and a 0.78 log reduction in milk chocolate treated for 30 min. They concluded that the milk chocolate offered marked protection against microbial inactivation.

Many studies investigated the use of ultrasound techniques in combination with other antimicrobial methods to enhance microbial inactivation of foods. Lillard (1993) found that only a slight (<1 log) reduction in *Salmonella* counts on poultry skin after treatment with chlorine. Sonification of skin in a chlorine solution was the most

effective treatment in reducing the population of *Salmonella* by 2.44 to 3.44 log. She concluded that sonification seemed to detach cells which were attached or entrapped in poultry skin, therefore making *Salmonella* more susceptible to the sanitizer. Ordoňez et al (1984, 1987) reported that *Staphylococcus aureus* was much more susceptible to the combined effect of ultrasonic (20 kHz/160 W) and heat treatment than by either treatment separately. Compared with heat treatment alone, the simultaneous application of ultrasound and heat reduced the population by about 1 additional log. Garcia et al (1989) showed that ultrasonic treatment (20 kHz/150 W) of two strains of *B. subtillis* spores in distilled water or milk resulted in a very slight decrease in heat resistance; however, when ultrasonic and heat treatment were applied simultaneously, the D-value decreased to a greater degree. Seymour et al. (2001) investigated the decontamination of raw vegetables with ultrasound. They reported that the cleaning action of cavitation removed *S.* Typhimurium cells, making *Salmonella* more susceptible to chlorine.

Scouten and Beuchat (2001) investigated combined effects of chemical, heat and ultrasound (ranging from 38.5-40.5 kHz) treatment on killing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds. Treatment with ultrasound alone at 23^{o}C for 2 or 5 min reduced *Salmonella* and *E. coli* O157:H7 populations by about 0.75 log and 0.58 log, respectively. Treatment at 55^{o}C for 2 or 5 min caused significantly higher lethality to those pathogenic bacteria. However, germination was adversely affected after treatment at 55^{o}C in 5 min. They reported that treatment of seeds at 23 or 55^{o}C with 1% Ca $(OH)_2$ was most effective in reducing populations without inhibiting the germination. Treatment of seeds at 55^{o}C for 5 min caused a 3.95 log and 2.71 log reduction of *Salmonella* and *E. coli* O157:H7 populations, respectively.

Dielectric Heating--Radio Frequency

Radio frequency (RF) heating refers to the use of electromagnetic waves of certain frequencies to generate heat in a material (Zhao, 1999). The majority of foods contain a substantial proportion of water. When radio-frequency electric fields are applied to a food, dipoles in the water and in some ionic components attempt to orient themselves with the field. Such rapid oscillations of the water molecules produce heat. Advantages of radio frequency processing include uniform heating, rapid attainment of the desired process temperature, and sterilization of the product after packaging.

Application of radio-frequency energy to grains and seeds

Because of concern about the health hazards of chemical pesticides, the use of radio-frequency energy for controlling stored-grain insects has been investigated. Nelson and Whitney (1960) have shown that temperatures ranging from 60 to 65^{o}C, depending on the characteristics of the insects and host material, have controlled insect successfully. Radio-frequency treatments at 10 and 39 MHz necessary for insect control were not damaging to wheat germination or milling and baking qualities (Nelson and Whitney, 1960; Nelson1996). The biological effects of radio-frequency heating are mainly believed to be thermal; the nonthermal effects have never been demonstrated convincingly. Nelson and Walker (1961) investigated the RF treatment of *Ustilago nuda*-infected barley. Smut-infected seeds (at 8 and 11% moisture content) were treated at frequencies of 10 and 39 MHz and field intensities of 0.67 and 1.89 kV/cm. Results showed this treatment of soaked seeds reduced *Ustilago nuda* (from 25% infection to 0%); however, the seed germination was severally inhibited. Lozano et al. (1986) revealed that seed-borne pathogens were eradicated by 80% in cassava true seeds after treatment with microwave heating (2040 MHz for 77^{o}C, 120 sec) without damaging the viability of seeds. Reddy et al. (1998) found that

microwave heating reduced seedborne populations of the pathogen *Fusarium graminerarum* by about 30% without reducing the wheat seed germination. Cwiklinski and Hőrsten(2001) investigated the effect of RF heating on *F. graminerarum* in wheat seeds. Radio-frequency treatment of seeds (at 14 and 16% moisture content) with frequencies at 27, 12 MHz, at 65 and 70^{o}C, respectively, for 500 sec eradicated the seedborne pathogen while maintaining germination.

Hard seed, a common condition found in alfalfa seeds, lowers the acceptable level of crop stands for production. Two methods have been used to reduce the frequency of hard seed. Mechanical scarification, which has been used by seedsmen to reduce hard-seed percentage, produces deterioration in seeds if held for the next season. Scarification may also exacerbates the problem of killing human pathogenic bacteria that may be present due to the lodging of bacterial cells within crevices or cracks on seeds rendering sanitizers ineffective. The second method involves the use of radio-frequency dielectric heating to improve the germination rate of alfalfa seeds. Treatment of seed lots with RF energy has reduced high percentages of hard seeds without producing changes in the rate or appearance of the plant growth (Nelson and Wolf, 1964; Nelson et al., 1984). The effects of several factors (such as frequency, field intensity, and moisture content) on RF treatment of alfalfa seeds also have been investigated. Nelson and Wolf (1964) revealed that RF dielectric heating at frequencies of 5, 10, and 39 MHz had about the same efficacy in reducing hard-seed percentages in alfalfa seeds. Germination response appeared similar with different field intensities, but higher field intensities were preferred since exposure time was reduced (Nelson and Wolf, 1964). A temperature rise was the most obvious effect when seeds were treated. Stetson and Nelson (1972) found that the temperature of optimum treatment range was the same for seed initially at 23 or -18^{o}C. The

temperature range at which optimum germination occurs was also the same for each initial temperature. Holding seeds at elevated temperatures for an hour did not increase further hard-seed reduction after the optimum exposure was achieved (Stetson and Nelson, 1972). Effectiveness of RF treatment in reducing hard seed content increased as moisture content of seeds decreased (Nelson and Wolf, 1964).

REFERENCE

Andrew, W. H., P. B. Mislevic, C. R. Wilson, and V. R. Bruce. 1982. Microbial hazards associated with bean sprouting. J. Assoc. Off. Anal. Chem. 65:241-248.

Anonymous, June 2000, "Ultrasound," "Kinetics of microbial inactivation for alternative food processing technologies," U.S. Food and Drug Administration Center of Food Safety and Applied Nutrition, [Internet, WWW], ADDRESS: http://vm.cfsan.fda.gov/~comm/ift-us.html

Bari, M. L., H. Kusunoki, H. Furukawa, H. Ikeda, K. Isshiki, and T. Uemura. 1999. Inhibition of growth of *E. coli* O157:H7 in fresh radish (*Raphanus sativas L.*) sprout production by calcinated calcium. J. Food Prot. 62:128-132.

Besser, R. E., S. M. Lett, J. T. Weber, M. P. Doyle, T. J. Barrett, J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA. 269:2217-2220.

Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. J. Food Prot. 59:204-216.

Beuchat, L. R.1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. Int. J. Food Microbiol. 34:329-333.

Beuchat, L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization.

Beuchat, L. R., T. E. Ward, and C. A. Pettigrew. 2001. Comparison of chlorine and a prototype produce wash product for effectiveness in killing *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 64:152-158.

Brackett, R. E. 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes*. J. Food Sci. 50:999-1003.

Caetano-Anolles, G., G. Favelukes, and W. D. Bauer. 1990. Optimization of surface sterilization for legume seed. Crop. Sci. 30:708-712.

Callister, S. M., and W. A. Agger. 1987. Enumeration and characterization of *Aeromonas hydrophilia* and *Aeromonas caviae* isolated from grocery store produce. Appl. Environ. Microbiol. 53:249-253.

Center of Disease Control and Prevention. 1995. Outbreak of *Escherichia coli* O157:H7, Northwestern Montana. EPI-AID 95-98.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 associated with eating alfalfa sprouts - Michigan and Virginia, June-July 1997. Morbid. Mortal. Weekly Rep. 46:741-744.

Curra, H. R. and A. Tamsma. 1960. Some observation in ultraviolet irradiation of milk (centrfilmer process) with emphasis upon organoleptic effects and sporicidal efficiency. J. Dairy Sci. 43:410-412.

Cwiklinski, M. and D. von Hőrsten 2001. Effect of exposure to radio-frequency electric fields on *Fusarium graminerarum* in wheat seed. ASAE Paper No.01-6171. Am. Soc. Agric. Eng., St. Joseph, MI.

D'Aoust, J.-Y. 1997. *Salmonella* Species. p. 129-158. *In*: M. P. Doyle, L. R. Beuchat and T. J. Monteville (ed.), Food Microbiology: Fundamentals and Frontiers, American Society for Microbiology, Washington, D. C.

Delaquis, P. J., P. L. Sholberg and K. Stanich. 1999. Disinfection of mung bean seed with gaseous acetic acid. J. Food Prot. 62:953-957.

Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Intl. J. Food Microbiol. 12:289-302.

Doyle, M. P., and J. L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855-856.

Doyle, M. P., and O. D. Cliver.1990. *Escherichia coli*, p.213. *In* O.D. Cliver (ed.), Foodborne Disease. Academic Press, San Diego.

Doyle, M. P., T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7. p. 171-191. *In*: M. P. Doyle, L. R. Beuchat and T. J. Monteville (ed), Food Microbiology: Fundamentals and Frontiers, American Society for Microbiology, Washington, D. C.

Feng P. 1997. "A summary of background information and foodborne illness associated with the consumption of sprouts," Center of Food Safety and Applied Nutrition. Washington, [Internet, WWW], ADDRESS: http:// www. cfsan.fda.gov/~mow/sprouts.html

Fleischeman, G. J., C. Bator, R. Merker, and S. E. Keller. 2000. Hot water immersion to eliminate *Escherichia coli* O157:H7 on the surface of whole apples: thermal effects and efficacy. J. Food Prot. 64:451-455.

Garcia, M. L., J. Burgos, B. Sanz, and J. A. Ordoňez. 1989. Effect of heat and ultrasonic waves on the survival of 2 strains of *Bacillus subtilis*. J. Appl. Bacteriol. 67:619-628.

Gray, M. L., and A. H. Killinger. 1966. *Listeria monocytogenes* and listeria infections. Bacteriol. Rev., 30:309-382.

Gutierrez, E. 1997. Japan prepares as O157 strikes again. Lancet. 349:1156.

Hara-Kudo, Y., H. Konuma, M. Iwaki, F. Kasuga, Y. Sugita-Konish, Y. Ito, and S. Kumagai. 1997. Potential hazard of radish sprouts as a vehicle of *Escherichia coli* O157:H7. J. Food Prot. 60:1125-1127.

Harmon, S. M., D. A. Kautter, and H. M. Solomon. 1987. *Bacillus cereus* contamination of seeds and vegetable sprouts grown in a home sprouting kit. J. Food Prot. 50:62-65.

Heisick, J. E., F. M. Harrell, E. H. Perterson, S. McLaughlin, D.E. Wagner, I.V. Wesley, and J. Bryner. 1989. Comparison of four procedures to detect *Listeria* spp. in foods. J. Food Prot. 52:154-157.

Hilgren, J.D., and J.A. Salverda. 2000. Antimicrobial efficacy of a peroxyaxetic/octanoic acid mixture in fresh-cut-vegetable process water. J. Food Sci. 65:1376-1379.

Hughes, D. E., and W. L. Nyborg. 1962. Cell disruption by ultrasound. Science. 138:108-114s.

Itoh, Y., Y. Sugita-Konishi, F. Kasuga, M. Iwaki, Y. Hara-Kudo, N. Saito, Y. Noguchi, H. Konuma, and S. Kumagai. 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl. Environ. Microbiol. 64:1532-1535.

Jackson, L. A. 1998. Overview of outbreaks of foodborne illness associated with fresh fruits and vegetables. In: Symposium on fresh fruits and vegetables: Food safety challenges, Institute of Food Technologists and the National Center for Food Safety and Technology, Rosemont, IL, May 12-14.

Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl. Environ. Microbiol. 62:2212-2215.

Jay, J. M. 1992. Foodborne listeriosis, p. 478-506. *In*: Modern Food Microbiology. 5th edition. Van Nostrand, New York.

Jerngklinchan, J., and K. Sartanu. 1993. The occurrence of salmonellae in bean sprouts in Thailand. Southeast Asian J. Trop. Med. Public Health. 24:114-118.

Juven, B. J., and M. D. Pierson. 1996. Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation. J. Food Prot. 59:1233-1241.

Kurtzweil, P. 1999. Questions keep sprouting about sprouts. FDA Consumer 33 (1):18-22.

Lang, M. M., B. H. Ingham, and S. C. Ingham. 2000. Efficacy of novel organic acid and hypochlorite treatments for eliminating *Escherichia coli* from alfalfa seeds prior to sprouting. Int. J. Food Microbiol. 58:73-82.

Lee, B. H., S. Kermasha, and B. E. Baker. 1989. Thermal, ultrasonic and ultraviolet inactivation of *Salmonella* in thin films of aqueous media and chocolate. Food Microbiol. 6:143-152.

Lillard, H. S. 1993. Bacterial effect of chlorine on attached Salmonellae with and without sonification. J. Food Prot. 56:716-717.

Lovett, J. 1989. *Listeria monocytogenes*, p. 283-310. *In*: Doyle, M. P. (ed.), Foodborne pathogens, Marcel Dekker, New York.

Lozano, J. C., R. Laberry, and A. Bermudez. 1986. Microwave treatment to eradicate seedborne pathogens in cassava true seed. J. Phytopathol. 117:1-8.

Mahon B.E., A. Ponka, W.N. Hall, K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M. A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infection caused by alfalfa sprouts grown from contaminated seeds. J. Infect. Dis. 175:876-882.

Marth, E. H., and J. Bahk. 1990. Listeriosis and *Listeria monocytogenes*. p.247-257. *In*: Foodborne Disease. Academic Press, San Diego.

Mazzotta, A. S. 2001a. Heat resistance of *Listeria monocytogenes* in vegetables: evaluation of blanching process. J. Food Prot. 64:385-387.

Mazzotta, A. S., 2001b. Thermal inactivation of stationary-phase and acid adapted *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in fruit juice. J. Food Prot. 64:315-320.

National Advisory Committee on Microbial Criteria for Foods. 1999. Microbial safety evaluation and recommendations on seed sprouts. Int. J. Food Microbiol. 52:123-153.

Neergaard, P. 1977. Seed pathology. Vol. 1, p. 118-146, The MacMillan Press, Ltd., London.

Nelson S. O., L. E. Stetson, and W. W. Wolf. 1984. Long-term effects of RF dielectric heating on germination of alfalfa seed. Trans. ASAE 27(1):255-258.

Nelson, S. O. 1996. Review and assessment of radio-frequency and microwave energy for stored-grain insect control. Trans. ASAE 39(4):1475-1484.

Nelson, S. O., and E. R. Walker. 1961. Effects of radio-frequency electrical seed treatment. Agric. Eng. 42(12):688-691.

Nelson, S. O., and W. K. Whitney. 1960. Radio-frequency electric fields for stored grain insect control. Trans. ASAE 3(2): 133-137, 144.

Nelson, S. O., and W. W. Wolf. 1964. Reducing hard seed in alfalfa by radio frequency electrical seed treatment. Trans. ASAE 7(2):116-119, 122.

Nguyen-The, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition. 34(4):371-401.

O'Mahony, M., J. Cowden, B. Smyth, D. Lynch, M. Hall, B. Rowe, E.L. Teare, R. E., Tettmar, A. M. Rampling, M. Coles, R. J. Gilbert, E. Kingcott, and L. R. Bartlett.

1990. An outbreak of *Salmonella* Saint-Paul infection associated with bean sprouts. Epidemiol. Infect. 104:229-235.

Ordoňez, J. A., B. Sanz, P. E. Hernandez, and P. Lopez-Lorenzo. 1984. A note on the effect of combined ultrasonic and heat treatments on the survival of thermoduric streptococci. J. Appl. Bacteriol. 56:175-177.

Ordoňez, J. A., M. A. Aguilera, M. L. Garcia, and B. Sanz. 1987. Effect of combined ultrasonic and heat treatments (thermoultrasonication) on the survival of a strain of *Staphylococcus aureus*. J. Dairy Res. 54:61-67.

Park, C.C., and D.W. Sanders. 1990. Source of *Klensiella pneumoniae* in alfalfa and mung bean sprouts and attempts to reduce its occurrence. J. Can. Inst. Sci. Technol.: 4:189-192.

Patterson, J. E., and M. J. Woodburn. 1980. *Klebsiella* and other bacteria on alfalfa and bean sprouts at the retail level. J. Food Sci. 45:492-495.

Perinas, V. and Guiraud, J. P. 1997. Disinfection of rice seeds prior to sprouting. J. Food Sci. 62:611-615.

Perinas, V. and Guiraud, J. P. 1998. Control of microbial growth on rice sprouts. Int. J. Food Sci. Tech. 33:297-305.

Pezzino, G., Miller, C., Flahart, R. and Potsic, S. R., 1998. A multi-state outbreak of *Salomonella* serotypes Infantitis and Anatum-Kansas and Missouri, 1997. Kansas Medicine 98(3):10-12.

Portnoy, B.L., J.M. Goeffert, and S.M. Harman. 1976. An outbreak of *Bacillus cereus* food poisoning resulting from contaminated vegetable sprouts. Am. J. Epidemiol. 103:589-594.

Puohiniemi, R., T. Heisknen, and A. Siitonen. 1997. Molecular epidemiology of two international sprouts-borne *Salmonella* Outbreaks. J. Clinical Microbiol. 35:2847-2491.

Reddy, M. V. B., G. S. V. Raghavan, A. C. Kushalappa, and T. C. Paulitz. 1998. Effect of microwave treatment on quality of wheat seeds infected with *Fusarium graminerarum*. Agric. Eng. Res. 71:113-117.

Scouten A. J., and L. R. Beuchat. Combined effects of chemical, heat, and ultrasound, treatments to kill *Salmonella* and *Escherichia coli* O157: H7 on alfalfa seeds. J. Food Prot. (In press).

Seymour, I. J., D. Burfoot, R. L. Smith, L. A. Cox, and Lockwood, A. 2001. Ultrasound decontamination of minimally processed fruits and vegetables. Int. J. Food Sci. Tech. (In press).

Steele, B. T., N. Murphy, G. S. Arbus, and C. P. Rance. 1982. An outbreak of hemolytic uremic syndrome associated with ingestion of fresh apple juice. J. Pediatr. 101:963-965.

Stetson, L. E., and S. O. Nelson. 1972. Effectiveness of hot-air, 39-MHz dielectric, and 2450-MHz microwave heating for hard-seed reduction in alfalfa. Trans. ASAE 15(3):530-535.

Taormina, P. J., and L.R. Beuchat. 1999. Comparison of chemical treatments to eliminated Enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 62:318-324.

Taormina, P. J., L.R. Beuchat, and R. Slusker. 1999. Infections associated with eating seed sprouts: An international concern. Emerg. Infect. Dis. 5:629-634.

Vollmer, A. C., E. C. Everbach, M. Halpern, and S. Kwakye. 1998. Bacterial stress response to 1-megahertz pulsed ultrasound in the presence of microbubbles. Appl. Environl. Microbiol. 64:3927-3931.

Weissinger, W. R., and L. R. Beuchat. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. J. Food. Prot. 63:1475-1482.

Zhao, T., M. P. Doyle, and R. E. Besser. 1993. Fate of enterohemorrhagic *Escherichia coli* O157: H7 in apple cider without preservatives. Appl. Environ. Microbiol. 59:2526-2530.

Zhao, Y. 2000. Using capacitive (radio frequency) dielectric heating in food processing and preservation-a review. J. Food Process Eng. 23:25-55.

CHAPTER 2

EFFICACY OF ULTRASOUND IN COMBINATION WITH HEAT AND CHEMICAL SOLUTIONS IN KILLING *SALMONELLA* ON ALFALFA SEEDS[1]

[1]Lu, C.-Y., Harrison, M. A., Beuchat, L. R. To be submitted to Journal of Food Protection

ABSTRACT

Since 1995, raw sprouts have been implicated with foodborne illness outbreaks of *Salmonella*. The efficacy of combined treatments of various chemical solutions, heat and ultrasound in killing *Salmonella* on alfalfa seeds prior to sprouting was investigated. Combined treatments of heat and sanitizers were effective in killing *Salmonella* on alfalfa seeds. Ultrasound treatment at 38.5-40.5 kHz in combination with heat and sanitizers did not appear to substantially add to the overall lethal effect. Ultrasound treatment of alfalfa seeds with 330 ppm Tsunami® 200 at 23°C for 2 or 5 min reduced the population of *Salmonella* by about 2 log CFU/g. Sanova® (1200 ppm acidified sodium chlorite) reduced the population of *Salmonella* by about 3 and 4 log CFU/g at 23°C and 55°C, respectively. Ultrasound treatment with 1% $Ca(OH)_2$ at 55°C for 5 min, 1% $Ca(OH)_2$ plus 1% Tween 80 at 23 or 55°C for 2 or 5 min, and 8% H_2O_2 at 23°C for 5 min and 55°C for 2 min reduced the population of *Salmonella* by at least 4.33 log CFU/g to undetectable levels (< 1 log CFU/g) without inhibiting the seed germination rate. However, none of these treatments eliminated the population of *Salmonella* on alfalfa seeds completely as evidenced by the recovery of *Salmonella* in an enrichment culture.

INTRODUCTION

Spouts are usually eaten raw and are considered as a healthy food because of their high nutritional value (Feng, 1997). However, a number of foodborne illness outbreaks have been associated with consumption of sprouts. During sprouting, warm temperatures, high moisture environment, release of nutrients by the seeds, and enzymatic factors support the growth of pathogenic bacteria (Hara-Kudo 1997; Patterson, 1980). *Salmonella* species have been held responsible to most sprouts-associated outbreaks. In

1988, beansprouts imported mainly from Australia and Thailand were implicated in an outbreak of 143 cases of *Salmonella* Saint-Paul in the United Kingdom (O'Mahnony et al., 1990). In 1995-1996, outbreaks in seven states in the U.S., and in Denmark and Canada were linked to *S*. Newport contaminated alfalfa sprouts. An epidemiological study showed the sources of these outbreaks were from contaminated seeds (NACMCF, 1999; Aabo and Baggesen, 1997). In 1996, an outbreak of approximately 450 cases of illness linked to consumption of alfalfa sprouts contaminated with *S*. Montevideo and *S*. Meleagridis was due to the unsanitary sprouting process, including practices such as fertilization of alfalfa fields with animal manure, use of canal water for watering crops, and use of unclean vehicles for carrying alfalfa seeds (NACMCF, 1999).

The most likely contamination sources of sprout-associated outbreaks are seeds (Puohiniemi et al., 1997; CDC, 1997; Mahon et al., 1997). Sanitizing is more effective for eliminating microorganisms on seeds than on sprouts because of lower microbial loads and organic materials present on the seeds (Hara-Kudo et al., 1997; Itoh et al., 1998; Caetano-Anolles et al., 1990). Washing with chlorine water is a widely used industry practice for decontamination of fresh produce. Jaquette et al. (1996) found that chlorine treatment of alfalfa seeds at 100-1,010 μg/ml for 5 or 10 min reduced the population of *S.* Stanley by about 1.5 logs. Treatment of seeds in a 2,040 μg/ml chlorine solution reduced the population of *S*. Stanley by 1 to 2 log CFU/g to an undetectable level (< 1 CFU/g). Treatment of alfalfa seeds with 20,000 ppm free chlorine $Ca(OCl)_2$ for 10 min eliminated 2 log CFU/g of *Salmonella*; treatment with 2,000 ppm $NaClO_2$ was less effective in reducing the population of *Salmonella* on alfalfa seeds (Weissinger and Beuchat, 2000).

Treatment of alfalfa seeds with 8% and 10% hydrogen peroxide for 10 min resulted in more than a 3 log reduction in the population of *Salmonella* without significantly inhibiting the germination; however, the presence of *Salmonella* was revealed by the subsequent enrichment (Beuchat, 1997; Weissinger and Beuchat, 2000). Treatment of alfalfa seeds with 1% $Ca(OH)_2$ and 1% calcinated calcium caused reduction in the population of *Salmonella* by about 3 log CFU/g. Treatment with 1% $Ca(OH)_2$ plus 1% Tween 80 resulted in an additional reduction of 1.3 log CFU/g compared to 1% $Ca(OH)_2$ treatment alone (Weissinger and Beuchat, 2000). Treatment of mung bean seeds with 242 µl gaseous acetic acid per liter of air for 12 h at 45°C reduced the population of *S.* Typhimurium by approximately 5 log CFU/g. *Salmonella* was not detected by enrichment culture (Delaquis et al., 1999). Based on previous seed decontamination studies, elimination of *Salmonella* on alfalfa seeds intended for sprouting has proven to be difficult. This might be caused by the pathogenic bacteria being trapped in the seed crevices or cracks, making the sanitizers unable to contact the viable cells.

The use of ultrasound techniques in combination with other antimicrobial methods can enhance microbial inactivation on foods. Sonification could intracellularly inactivate microorganisms through cavitation or detach the entrapped microbial cells in the food tissues rendering microorganisms more susceptible to sanitizer (Lillard, 1993; FDA, 2000; Seymour et al., 2001). Scouten and Beuchat (2001) investigated combined effects of chemical, heat and ultrasound (ranging from 38.5-40.5 kHz) treatment on killing *Salmonella* on alfalfa seeds. They reported that ultrasound treatment of seeds at 55°C in combination with 1% $Ca(OH)_2$ was the most effective in reducing populations (3.95 log reduction of *Salmonella*) without inhibiting the seed germination. The objective of this

study was to determine the efficacy of additional combinations of chemicals, heat, and ultrasound treatments in killing *Salmonella* on alfalfa seeds.

MATERIALS AND METHODS

Strains

Five serotypes of *Salmonella* were used. *Salmonella* serotypes Anatum (H3536), Cubana (H7976) and Stanley (H1256) were all from alfalfa sprout-associated outbreaks. *S*. Gaminara (F2712) and *S*. Montevideo (G4639) were from orange juice and tomato-associated outbreaks, respectively. All were obtained from the culture collection at the Center for Food Safety, University of Georgia, Griffin, GA.

Preparation of inocula

Salmonella were grown in tryptic soy broth (Difco, Sparks, MD, USA) supplemented with 0.05 g/L of nalidixic acid (Sigma, St. Louis, MO, USA) (mTSB) at 37°C. Cultures were transferred (one loopful to 10 ml of mTSB) three times at 24-h intervals immediately preceding use as inocula for alfalfa seeds.

Inoculation of alfalfa seeds

Six ml of 24-h mTSB culture of each serotype of *Salmonella* were combined with 1 L of sterile deionized water (23±2°C). Alfalfa seeds (Mumm's Sprouting Seeds, Parkside, SK) (1kg, 23±2°C) were added to the cell suspension and gently stirred for 1 min. The suspensions containing the seeds were drained into a pan through a double layer of sterile cheese cloth supported by a wire screen elevated approximately 5 cm above the work surface of a laminar flow hood. The seeds were spread in a layer 0.5 cm thick, dried for 72 h, placed in a stomacher bag (Seward Stomacher® bags, London, UK), and stored at 5°C for 8 days before use. On the day of use,water activity of a seed sample was

measured with a water activity meter (Decagon, Aqualab series 3, Pullman, WA, USA). In addition, *Salmonella* was enumerated from 10 g portions of seeds. To each bag, 40 ml of Dey-Engley (D/E) neutralize broth (Difco), was added prior to stomaching for 60 sec at medium speed. DE wash broth was spiral plated (Morton et al, 2000; Spiral Biotech, Bethesda, MD, USA) on tryptic soy agar (Difco) supplemented with 0.05 g/L nalidixic acid (TSAN). After incubation at 37°C for 24 h, colonies formed on TSAN were counted.

Preparation of chemical treatment solutions

Five chemical treatments were evaluated for effectiveness in eliminating *Salmonella* on alfalfa seeds: 1% (wt/vol) $Ca(OH)_2$ (J. T. Baker, Pillipsburg, NJ, USA) in sterile deionized water; 1% (wt/vol) $Ca(OH)_2$ plus 1% (vol/vol) Tween 80 (ICN Biomedicals, Aurora, Ohio, USA) in sterile deionized water; 8% (vol/vol) H_2O_2 (VWR, West Chester, PA) in sterile deionized water; 330 ppm Tsunami® 200 (Ecolab, St. Paul, MN, USA); and 1200 ppm Sanova® (Alcide, Redmond, WA, USA). All chemical treatment solutions were prepared within 30 min of use. Peroxyacetic acid concentration in Tsunami® solution were determined using a Tsunami® test kit (Ecolab). The temperature of solutions and seeds was 23±2°C when treatments were applied. Treatment of inoculated seeds in 1% peptone water (Difco) served as a control.

Procedures for treating seeds

Water in an ultrasound cleaner bath (Aquasonic Cleaner Bath, model 250D, VWR Scientific, West Chester, PA, USA) was adjusted to 23 or 55°C. Treatment solutions and water in the ultrasound cleaner bath were degassed for 10 min immediately before seed decontamination experiments were done. The frequency of ultrasound fluctuated between 38.5 and 40.5 kHz and the set temperature shifted ± 2°C during treatment of seeds.

The efficacy of treatment of seeds was determined. Twenty ml of chemical solutions or 1% peptone water (control) were added to 5 g of inoculated seeds in stomacher bags (Seward) before placing the bags in the ultrasound cleaner bath at either 23 or 55^{o}C such that the level of the solution was below the level of water. Seeds were treated with ultrasound either for 2 or 5 min.

Treatment of seeds with chemical solutions stated above, with exception of 1% (wt/vol) Ca $(OH)_2$ plus 1% (vol/vol) Tween 80 (ICN Biomedicals), and heat without ultrasound at the same temperature for the same time was also tested.

Microbiological analyses

Immediately after treatment for either 2 or 5 min, solutions were decanted from treated seeds and 20 ml of DE neutralize broth (Difco) were added to the inoculated seeds. Approximately 100 seeds were removed from each stomach bag to determine germination percentage before the seed and DE neutralize broth were pummeled in a stomacher for 1 min. Stomachates were spiral plated (Morton et al, 2000; Spiral Biotech.) on TSAN. Plates were incubated at 37^{o}C for 24 h and presumptive colonies were counted. Presumptive colonies formed on TSAN were identified by streaking randomly selected colonies on bismuth sulfite agar (Difco) supplemented with 0.05 g/L nalidixic acid (Sigma) (BSAN). Twenty milliliters of double-strength lactose broth supplemented with 100 μg/ml nalidixic acid, were added to pummeled seed/DE broth mixture and then incubated at 37^{o}C for 24 h. In the event that the number of colonies formed on spiral TSAN plates was below the level of detection, the enrichment cultures were streaked on BSAN. These plates were incubated at 37^{o}C for 24 h to examine the presence of presumptive colonies of *Salmonella*. Identification of randomly selected presumptive

colonies formed on all BSAN was confirmed by MicroID (Organon Teknik Co., Durham, NC, USA).

Statistical analysis

Three replicate experiments for each set of experimental parameters were conducted. Data were subjected to the SAS for analysis of variance (ANOVA) and Duncan's multiple range tests.

RESULTS AND DISCUSSION

The results shown in Table 2.1 are populations of *Salmonella* on alfalfa seeds treated with the combination of various chemical solutions, heat and ultrasound. Ultrasound treatment alone only has a very slight effect on eliminating the population of *Salmonella* on alfalfa seeds (<1 log). Treatment with ultrasound in combination with heat (55^{o}C) did not reduce the germination percentage of seeds with exception of treatments with 8% H_2O_2 and 1200 ppm Sanova for 5 min. Treatment at 55^{o}C was more lethal to *Salmonella* than treatment at 23^{o}C, regardless of the presence or absence of chemical solutions. Treatment of alfalfa seeds with 1% $Ca(OH)_2$ at 55^{o}C for either 2 or 5 min reduced the populations of *Salmonella* by 4.33 log CFU/g without inhibiting the germination rate. The water activity of alfalfa seeds used in various combination of treatment in this study was consistent (0.42 a_w). In this study, treatment with 1% $Ca(OH)_2$ at 23^{o}C for 5 min reduced an additional 1.96 log CFU/g of *Salmonella* populations compared to the same treatment used in another study (Scouten and Beuchat, 2001). The efficacy of seed sanitation may differ between seed lot and seed cultivars. The reduction of *Salmonella* populations after treatment at 55^{o}C for 5 min is similar to the results observed in Scouten and Beuchat's (2001) study.

Regardless of treatment time and temperature, treatment with 1% $Ca(OH)_2$ plus 1% Tween 80 was the most effective in reducing the population of *Salmonella* (≥ 4.33 log CFU/g) without significantly ($P \leq 0.05$) inhibiting the germination rate. Another study also observed a similar reduction (4.05 log) after being treated with the combination of 1% $Ca(OH)_2$ plus 1% Tween 80 and ultrasound at 55°C for 5 min; however, the seed viability was inhibited (Scouten and Beuchat, 2001). This study and that of Weissinger and Beuchat (2000), which did not apply ultrasound, did not reduce germination percentage after treatment with 1% $Ca(OH)_2$ plus 1% Tween 80. Taormina and Beuchat (1999) found that the same chemical treatment of seeds caused different effects on the germination rate from two different seed lots (one was inhibited, the other was not).

Treatment of seeds with 8% hydrogen peroxide at 23°C for 5 min or 55°C for 2 or 5 min reduced the population of *Salmonella* by ≥ 4.33 log CFU/g (Table 2.1). The reduction from this study is about 1 log more than the reduction observed by Weissinger and Beuchat (2000), who found 8% H_2O_2 treatment for 10 min reduced the population of *Salmonella* by 3.22 log CFU/g. A 10% H_2O_2 treatment of alfalfa seeds for 30 sec reduced population by 3.57 log CFU/g without inhibiting the germination rate (Beuchat, 1997). Part of this difference between the studies may be due to the differences in the initial *Salmonella* populations on the seeds. In this study, the germination percentage of seeds was inhibited after being treated with 8% H_2O_2 at 55°C for 5 min.

Treatment with Tsunami® 200 was less effective in reducing the *Salmonella* population compared to treatments with other chemical solutions at the same temperature and for the same time. Treatment of seeds with 330 ppm Tsunami® 200, which contains 25 ppm peroxyacetic acid, at 23°C for 2 or 5 min reduced the *Salmonella* population by

about 2 log CFU/g. This is in agreement with the reduction observed by Scouten and Beuchat (2001) who applied 160 ppm of Tsunami® on seeds. Treatment of seeds at 55°C caused a slightly higher reduction of populations without inhinbiting the germination rate.

Sanova® (1200 ppm acidified sodium chlorite) reduced the population of *Salmonella* by about 3 and 4 log CFU/g at 23°C and 55°C, respectively. Weissinger and Beuchat (2000) reported a 1.43 log CFU/g reduction of *Salmonella* populations after treatment with acidified sodium chlorite treatment for 10 min at room temperature (without ultrasound). A 1200 ppm acidified sodium chlorite at 21°C for 0.5 min (without ultrasound) reduced the population of *E. coli* O157:H7 on alfalfa seeds by 2.87 log CFU/g (Taormina and Beuchat, 1999). The slightly higher level of microbial inactivation in this study might be contributed to the effectiveness of ultrasound treatment.

To determine whether or not the ultrasound treatment contributed to the overall reduction in *Salmonella* population on tne seeds, inoculated seeds were exposed to chemical and heat treatments without ultrasound. As with the combined ultrasound, chemical and heat treatment, 1% $Ca(OH)_2$ and 8% H_2O_2 were effective in reducing significantly ($P \leq 0.05$) the *Salmonella* population by over 4 logs at either 23 or 55°C (Table 2.2). Tsunami 200 and Sanova at both temperatures with ultrasound also reduced the *Salmonella* population significanly, although they were less effective than 1% $Ca(OH)_2$ and 8% H_2O_2. Additional evidence of the effectiveness of the chemical solutions is provided by the lethal effect on the *Salmonella* in the rinse. All four chemical solutions tested reduced the *Salmonella* population in the rinse solutions to undectable levels by spiral plating procedures. Thus, the majority, if not all, of the lethal effect observed

appears to be due to the exposure to the chemical and heat with ultrasound contributing little to the overall effect.

Overall, combined treatments of heat and sanitizers were effective in killing *Salmonella* on alfalfa seeds. Ultrasound treatment at 38.5-40.5 kHz in combination with heat and sanitizers did not substantially appear to add to the overall lethal effect.

Table 2.1. Combined effect of chemical, heat, and ultrasound (38.5-40.5 KHz) treatments in killing *Salmonella* on alfalfa seeds

Chemical	Temp (°C)	Time (min)	Population (log CFU/g)[b]	E[a]	Reduction (log CFU/g)[c]	Germination (%)[b]
Water	23	0	5.03 a	_[d]		79 a
		2	4.70 b	_	0.33	78 a
		5	4.54 c	_	0.49	76 a
	55	0	5.03 a	_		79 a
		2	4.18 b	_	0.85	77 a
		5	3.25 c	_	1.78	76 a
$Ca(OH)_2$ (1%)	23	0	5.03 a	_		79 a
		2	0.70 b	_	4.33	79 a
		5	0.80 b	_	4.23	81 a
	55	0	5.03 a	_		79 a
		2	<0.70 b	3	>4.33	81 a
		5	<0.70 b	3	>4.33	81 a
$Ca(OH)_2$ (1%) + Tween 80 (1%)	23	0	5.03 a	_		79 a
		2	<0.70 b	3	>4.33	74 a
		5	<0.70 b	3	>4.33	76 a
	55	0	5.03 a	_		79 a
		2	<0.70 b	3	>4.33	87 b
		5	<0.70 b	3	>4.33	78 a
H_2O_2 (8%)	23	0	5.03 a	_		79 a
		2	0.90 b	_	4.13	76 a
		5	<0.70 b	3	>4.33	76 a

(continued)

Table 2.1. (Continued)

Chemical	Temp (°C)	Time (min)	Population (log CFU/g)[b]	E[a]	Reduction (log CFU/g)[c]	Germination (%)[b]
H_2O_2 (8%)	55	0	5.03 a	_[d]		79 a
		2	<0.70 b	3	>4.33	82 a
		5	<0.70 b	3	>4.33	68 b
Tsunami 200 (330 ppm)	23	0	5.03 a	_		79 a
		2	3.06 b	_	1.97	80 a
		5	3.01 b	_	2.02	79 a
	55	0	5.03 a	_		79 a
		2	1.10 b	_	3.93	82 a
		5	<0.70 b	3	>4.33	80 a
Sanova (1200 ppm)	23	0	5.03 a	_		79 a
		2	1.80 b	_	3.23	82 a
		5	2.14 b	_	2.89	80 a
	55	0	5.03 a	_		79 a
		2	1.31 b	_	3.72	82 b
		5	0.80 b	_	4.23	74 c

[a.] Number of samples of three analyzed that were positive for *Salmonella* as determined by enrichment.

[b.] Mean values for different time interval within chemical solution and temperature treatements with different letters are significant ($P \leq 0.05$) different.

[c.] Within chemical treatment and temperature, $\log_{10}$ reduction in number of *Salmonella* compared to no treatment (0 min).

[d.] —: Enrichment broths were not checked since enumeration of *Salmonella* was possible.

Regardless, the combined treatments did not significantly ($P \leq 0.05$) reduce seed germination rates, with an exception of treatment with 8% H_2O_2 at 55°C for 5 min. Otherwise the seed viability was not adversely affected by ultrasound treatment. Jaquette et al. (1996) also reported that dipping alfalfa seeds in water at 54, 57, or 60°C for 5 min did not substantially reduce the germination percentage. Treatment with 1% $Ca(OH)_2$ plus 1% Tween 80 at 23 or 55°C, 1% $Ca(OH)_2$ and 8% H_2O_2 at 55°C for either 2 or 5 min reduced the population of *Salmonella* by 4.33 log CFU/g to an undetectable level; however, positive presumptive colonies were recovered after the enrichment procedure. The failure in eliminating pathogen populations on alfalfa seeds intended for sprouting was also reported in other studies (Taromina and Beuchat, 1999; Weissinger and Beuchat, 2000; Beuchat et al, 2001; Scouten and Beuchat, 2001). This might be due to *Salmonella* cells entrapped in the cracks or crevices on the seed coats which makes them inaccessible to the sanitizer. Sanitizing efficacy varies between seed lots or between seeds within each seed lot. Wrinkled alfalfa seeds, which were damaged by insects making holes in the alfalfa pods, allowing more

bacteria cells to lodge inside the pods, compared to smooth alfalfa seeds, makes sanitization more difficult. The reduced lethality of active chlorine after contact with organic matter might be another part of the reason for failure of sanitization. Sanitizing could be improved if alfalfa seeds were sorted by smooth of wrinkled types before application of chemical and physical treatments.

Table 2.2. Combined effect of chemical and heat treatments in killing *Salmonella* on alfalfa seeds

Chemical	Temp (^oC)	Time (min)	Population (log CFU/g)[b]	Reduction (log CFU/g)[a]	Populations in Rinse solution (log CFU/ml)
Water	23	0	4.98 a		
		2	4.58 b	0.4	3.57
		5	4.58 b	0.4	3.50
	55	0	4.98 a		
		2	4.29 b	0.69	2.76
		5	4.09 b	0.89	3.22
$Ca(OH)_2$ (1%)	23	0	4.98 a		
		2	<0.70 b	>4.28	<0.70
		5	<0.70 b	>4.28	<0.70
	55	0	4.98 a		
		2	<0.70 b	>4.28	<0.70
		5	<0.70 b	>4.28	<0.70
H_2O_2 (8%)	23	0	4.98 a		
		2	<0.70 b	>4.28	<0.70
		5	<0.70 b	>4.28	<0.70
	55	0	4.98 a		
		2	<0.70 b	>4.28	<0.70
		5	<0.70 b	>4.28	<0.70
Tsunami 200 (330 ppm)	23	0	4.98 a		
		2	2.90 b	2.08	<0.70
		5	3.38 b	1.6	<0.70

(continued)

Table 2.2. (Continued)

Chemical	Temp (°C)	Time (min)	Population (log CFU/g)[b]	Reduction (log CFU/g)[a]	Populations in Rinse solution (log CFU/ml)
Tsunami 200 (330 ppm)	55	0	4.98 a		
		2	1.78 b	3.20	<0.70
		5	<0.70 c	4.28	<0.70
Sanova (1200 ppm)	23	0	4.98 a		
		2	2.43 b	2.55	<0.70
		5	2.26 b	2.72	<0.70
	55	0	4.98 a		
		2	<0.70 b	4.28	<0.70
		5	<0.70 b	4.28	<0.70

[a.] Within chemical treatment and temperature, $\log_{10}$ reduction in number of *Salmonella* compared to no treatment (0 min).

[b.] Mean values for different time interval within chemical solution and temperature treatements with different letters are significant ($P \leq 0.05$) different.

REFERENCE

Aabo, S. and D. L. Baggeson. 1997. Growth of *Salmonella* Newport in naturally contaminated alfalfa sprouts and estimation of infectious dose in Danish *Salmonella* Newport outbreak due to alfalfa sprouts. In: Program and abstracts of *Salmonella* and salmonellosis `97. Nice, France, p. 425-426.

Beuchat, L. R.1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. Int. J. Food Microbiol. 34:329-333.

Caetano-Anolles, G., G. Favelukes, and W. D. Bauer. 1990. Optimization of surface sterilization for legume seed. Crop. Sci. 30:708-712.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 associated with eating alfalfa sprouts - Michigan and Virginia, June-July 1997. Morbid. Mortal. Weekly Rep. 46:741-744.

Delaquis, P. J., P. L. Sholberg and K. Stanich. 1999. Disinfection of mung bean seed with gaseous acetic acid. J. Food Prot. 62:953-957.

Feng P. 1997. "A summary of background information and foodborne illness associated with the consumption of sprouts," Center of Food Safety and Applied Nutrition. Washington, [Internet, WWW], ADDRESS: http:// www. cfsan.fda.gov/~mow/sprouts.html

Hara-Kudo, Y., H. Konuma, M. Iwaki, F. Kasuga, Y. Sugita-Konish, Y. Ito, and S. Kumagai. 1997. Potential hazard of radish sprouts as a vehicle of *Escherichia coli* O157:H7. J. Food Prot. 60:1125-1127.

Itoh, Y., Y. Sugita-Konishi, F. Kasuga, M. Iwaki, Y. Hara-Kudo, N. Saito, Y. Noguchi, H. Konuma, and S. Kumagai. 1998. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Appl. Environ. Microbiol. 64:1532-1535.

Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl. Environ. Microbiol. 62:2212-2215.

Lillard, H. S. 1993. Bacterial effect of chlorine on attached Salmonellae with and without sonification. J. Food Prot. 56:716-717.

Mahon B.E., A. Ponka, W.N. Hall, K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M. A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infection caused by alfalfa sprouts grown from contaminated seeds. J. Infect. Dis. 175:876-882.

Morton, R. D. 2000. p.66. *In* Downes, F. P. and Ito, K. (ed.), Compendium of methods for the microbiological examination of foods. 4th ed., American Public Health Association. Washington DC, USA.

National Advisory Committee on Microbial Criteria for Foods. 1999. Microbial safety evaluation and recommendations on seed sprouts. Int. J. Food Microbiol. 52:123-153.

O'Mahony, M., J. Cowden, B. Smyth, D. Lynch, M. Hall, B. Rowe, E.L. Teare, R. E. Tettmar, A. M. Rampling, M. Coles, R. J. Gilbert, E. Kingcott, and L. R. Bartlett. 1990. An outbreak of *Salmonella* Saint-Paul infection associated with bean sprouts. Epidemiol. Infect. 104:229-235.

Patterson, J. E., and M. J. Woodburn. 1980. *Klebsiella* and other bacteria on alfalfa and bean sprouts at the retail level. J. Food Sci. 45:492-495.

Puohiniemi, R., T. Heisknen, and A. Siitonen. 1997. Molecular epidemiology of two international sprouts-borne *Salmonella* Outbreaks. J. Clinical Microbiol. 35:2847-2491.

Scouten A. J., and L. R. Beuchat. Combined effects of chemical, heat, and ultrasound, treatments to kill *Salmonella* and *Escherichia coli* O157: H7 on alfalfa seeds. (In press).

Seymour, I. J., D. Burfoot, R. L. Smith, L. A. Cox, and Lockwood, A. 2001. Ultrasound decontamination of minimally processed fruits and vegetables. Int. J. Food Sci. Tech. (In press).

Taormina, P. J., and L.R. Beuchat. 1999. Comparison of chemical treatments to eliminated Enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 62:318-324.

Weissinger, W. R., and L. R. Beuchat. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. J. Food. Prot. 63:1475-1482.

CHAPTER 3

APPLICATION OF DIELECTRIC HEATING IN KILLING HUMAN PATHOGENIC BACTERIA ON ALFALFA SEEDS[1]

[1]Lu, C.-Y., Harrison, M. A., Beuchat, L. R. To be submitted to Journal of Food Protection

ABSTRACT

Numerous outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infections has been associated with the consumption of contaminated alfalfa sprouts since 1995. Outbreaks of listeriosis associated with consumption of sprouts have yet to be documented; however, there are many chances for it to contaminate either seeds or sprouts. Radio-frequency dielectric heating, has been used to improve the germination percentage of alfalfa seed and to control insects in grains. The efficacy of dielectric heating to kill foodborne pathogens, *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes*, as affected by moisture content of seeds and various treatment time/temperature combinations, was investigated. Dielectric heating significantly ($P \leq 0.05$) reduced the pathogen populations without adversely affecting the germination percentage and vigor. Treatment of seeds with 6.5% moisture content for 20 sec to 89^{o}C reduced *E. coli* O157:H7 and *Salmonella* populations by 1.40 and 1.06 logs, respectively. Treatment of seeds with 6.4% moisture for 12 sec to 82^{o}C reduced *L. monocytogenes* populations by 0.89 logs.

INTRODUCTION

Since 1995, there have been numerous outbreaks of *Escherichia coli* O157:H7 and *Salmonella* infections in the U.S. and worldwide associated with the consumption of contaminated alfalfa sprouts (Ponka et al., 1995; CDC, 1997). Since the majority of sprouts-associated outbreaks are from contaminated seeds (Puohiniemi et al, 1997; CDC, 1997; Mahon et al, 1997), seed decontamination by various methods has been investigated. Treatment of seeds in water at 54^{o}C for 5 or 10 min resulted 1.6 log reduction of *S*. Stanley. However, treatment at >54^{o}C significantly reduced viability of

the seeds (Jaquette et al., 1996). Various aqueous chemical treatments of alfalfa seeds including rinses with chlorine (up to 20,000ppm), chlorine dioxide, hydrogen peroxide, trisodium phosphate, ethanol, peracetic acid and commercial produce wash solutions have been investigated, but none of these treatments eliminated *E. coli* O157:H7 and *Salmonella* on alfalfa seeds intended for sprouting (Jaquette et al, 1996; Beuchat, 1997; Taormina and Beuchat, 1999; Weissinger and Beuchat, 2000). The inability of sanitizers to reduce pathogenic bacteria might be due to the pathogenic bacteria entrapped in seed crevices or cracks rendering them inaccessible to sanitizers. Outbreaks of listeriosis associated with consumption of sprouts has yet to be documented; however, due to *Listeria monocytogenes* prevelence in nature, there are many chances for it to contaminate either seeds or sprouts. *L. monocytogenes* can grow at temperatures as low as 2 to 4^{o}C on a variety of produce, including sprouts (NACMCF, 1999; Lovett, 1989).

Dielectric heating, i.e., radio-frequency heating, has successfully controlled insects in grain (Nelson and Whitney, 1960). Radio-frequency heating also has been shown to eliminated seedborne plant pathogens in grain (Nelson and Walker, 1961; Cwiklinski and Hőrsten, 2001). Radio frequency heating has also been used to improve the germination of alfalfa seed lots by reducing the high percentages of hard seeds without producing changes in the rate or appearance of the plant growth (Nelson and Wolf, 1964; Nelson et al., 1984). Hard seed is a common condition in alfalfa which lowers the sprout yield because of lower germination. Mechanical scarification, which has been used to reduce hard-seed percentage, produces deterioration of germination if seeds are held for long periods and also exacerbates the problem of killing human pathogenic bacteria due to the

lodging of bacterial cells in crevices or cracks on seeds and rendering sanitizers less effective.

The objective of this study was to reduce populations of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on alfalfa seeds with dielectric heating while simultaneously enhancing germination and not adversely affecting vigor or sensory quality of sprouts. The efficacy of dielectric heating in killing pathogenic bacteria, as affected by moisture content of seeds and various treatment time/temperature combinations, was determined. The effect of treatments on seed germination percentage and vigor (overall appearance, color) was also investigated.

MATERIALS AND METHODS

Strains

Mixtures of five strains of each pathogen were separately inoculated onto alfalfa seeds. *E. coli* O157:H7 E0018 (calf feces), F4546 (patient from alfalfa sprout outbreak), H1730 (patient from lettuce outbreak), 932 (human fecal isolate), and 994 (salami), *Salmonella* serotypes Anatum (H3536), Cubana (H7976) and Stanley (H1256), all from alfalfa sprout-associated outbreaks, Gaminara (F2712) from orange juice and Montevideo (G4639) from a tomato-associated outbreak, and *L. monocytogenes* G 1091 (patient from coleslaw-associated outbreak), F8027 (celery), F8255 (peach and plum), F8369 (corn), and H0222 (raw potato) were available from the culture collection at the Center for Food Safety, University of Georgia, Griffin, GA.

Preparation of inocula

E. coli O157:H7 and *Salmonella* were grown in tryptic soy broth (TSB) (Difco, Sparks, MD, USA) supplemented with 0.05 g/L nalidixic acid (Sigma, St. Louis, MO,

USA) at 37°C. *L. monocytogenes* was grown in tryptic phosphate broth (TPB) (Difco) supplemented with 0.05g/L nalidixic acid, also at 37°C. All strains were transferred three times at 24-h intervals immediately preceding use as inocula for alfalfa seeds.

Procedure for inoculation

Six milliliters (24-h culture) of each serotype were inoculated into 1 liter of sterile water (23±2°C). Alfalfa seeds (Mumm's Sprouting Seeds, Parkside, SK) (1kg) were immersed in each inoculum and gently stirred for 1 min. The seeds were separated from the cell suspension by pouring the mixture into a pan through a double layer of sterile cheesecloth which was supported by a wire screen elevated approximately 5 cm above the work surface of a laminar flow hood. The seeds were then spread in a layer approximately 0.5 cm thick and allowed to dry in the hood at 23 ±2°C for 48 to 96 hours to reach moisture levels between 6 and 11.8%. To reach less than 6% moisture content for inoculated seeds, they were transferred from the hood to an anhydrous calcium sulfate desiccator for about 2 days. Moisture contents (indicated on a wet basis) were determined by drying triplicate, 10 g-seed samples for 2.5 h at 130^{o}C in a forced-air oven (ASAE Standard Method). Inoculated dried seeds were then placed in stomacher 80 bags, sealed, and stored at 5°C until used in dielectric heating treatments.

Procedure for dielectric heating treatment

A 2.3 cm diameter x 5 cm high metal, closed bottom cylinder filled uniformly to the rim was used to portion inoculated seeds into polystyrene Althor Products P-3 boxes (Althor Products Company, Bethel, CT, USA). The 4.3 cm squared x 1.5 cm high P-3 boxes held approximately 25 g of seeds and were treated for specified times between parallel-plate electrode using a modified General Electric Model 4HD3B2 3KW

electronic dielectric heater operating at 39 MHz with field intensities ranging from 0.3 to 1.9 kV/cm. The moisture content of inoculated seeds ranged from 3.2% to 11.8%. For each experiment, a sequence of exposure times were selected that was expected to span the range from little effect to noticeable damage of seed viability. Immediately after radio-frequency heating exposure, the temperatures of the treated seeds were measured at the center of the boxes with a recording thermocouple potentiometer and extrapolation technique (Nelson and Whitney, 1960). The final temperatures produced by treatment ranged from 50 to 126^{o}C. Samples were cooled to room temperature in the P-3 boxes in which they were treated. Samples were then stored at 5^{o}C until microbiological analysis and germination rates were determined.

Analysis of treated seeds

Treated and control seeds (10 g) were placed in a stomacher 80 bag; 40 ml of 0.1% peptone water (Difco) were added to the seeds, and stomached (Tekmar, Cincinnati, OH, USA) for 1 min with normal speed. The peptone wash solutions of *E. coli* O157:H7 and *Salmonella* inoculated seeds were spiral plated (Morton, 2000; Spiral Biotech, Bethesda, MD. USA) on tryptic soy agar (Difco) supplemented with 0.05g/L nalidixic acid (TSAN). The peptone wash solutions of *L. monocytogenes* inoculated seeds were spiral plated on Listeria selective agar base (oxford formulation) supplemented with 0.05 g/L nalidixic acid (MOXN agar) (Oxoid, Hampshire, England). All plates were incubated at 37^{o}C, examined at 24 h (MOXN plates also examined at 48 h), and colony formining units were counted. Three presumptive colonies of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* from randomly selected and plates were streaked onto sorbitol MacConkey agar (SMACN) (Oxoid), bismuth sulfite agar (BSAN) (Difco), and MOXN

agar (Oxoid), respectively. All media were supplemented with 0.05 g/L nalidixic acid. Identification of colonies formed on plates after incubation at 37^{o}C for 24 h were confirmed by MicroID (Organon Teknik Co., Durham, NC, USA) and latex agglutination reaction (Oxoid) (for *E. coli* O157:H7 only).

Determination of seed germination percentage

For each heating trial, approximately 50 treated and control seeds were placed between two pieces of water-saturated blotting paper (Anchor Paper, St. Paul, MN, USA) in a 100 x 15mm plastic petri dish and placed in the dark at 21^{o}C for 7 days. The number of germinated seeds was counted and the percentage that germinated was calculated.

Sensory analysis of alfalfa sprouts

Sprouts from treated and untreated seeds, germinated in petri dishes for 4 days, were provided for sensory analysis. A panel of ten individuals from the department was used to evaluate the overall appearance and color of both treated and untreated sprouted seeds using a nine-point hedonic scale, with 1= dislike extremely, 5= neither like nor dislike, and 9= like extremely. Samples were assigned random three-digit codes and randomly presented to the panelists in plastic weigh boats under normal fluorescence room light in the laboratory.

Statistical analysis

Three replicate trials for each treatment were conducted. Data were subjected to the analysis of variance (ANOVA) and Duncan's multiple range test (SAS) to determine differences in populations of each test pathogen detected on alfalfa seeds as affected by various treatment and differences between sensory attributes of both treated and untreated sprouted seeds.

RESULTS AND DISCUSSION

The relationships between dielectric heating, exposure time, final seed temperature, germination rate and pathogenic populations for about the same heating rate (~3^{o}C/sec) are shown in Figures 1-3. The population of *Salmonella* on 6.5% moisture content alfalfa seeds was reduced by 2.42 log CFU/g after treatment for 26 sec at 103^{o}C; however, the germination rate of seeds was seriously inhibited (Fig. 3.1A). The optimum exposure of *Salmonella* inoculated seeds of 6.5% moisture without reduction of germination was 20 sec to 89^{o}C. This treatment significantly ($P \leq 0.05$) reduced the *Salmonella* populations by 1.06 log CFU/g without damaging the seed viability. For *Salmonella* inoculated seeds with 8.1% moisture, the population was reduced by 2.32 log after treatment for 22 sec at 100^{o}C, but again the seed viability was adversely affected (Fig. 3.1B). Without inhibiting the germination rate, the best exposure was for 16 sec to 85^{o}C which significantly reduced the population of *Salmonella* by 1.04 log CFU/g. The population of *E. coli* O157:H7 on alfalfa seeds (6.5% moisture content) was significantly reduced by 1.40 log CFU/g after treatment for 20 sec to 89^{o}C without compromising the seed viability (Fig. 3.2A). *E. coli* O157:H7 population on alfalfa seeds at 8.1% moisture was significantly reduced by 0.81 log CFU/g after treatment for 16 sec to 86^{o}C (Fig. 3.2B). Without regard for seed viability, the population of *E. coli* O157:H7 was reduced by 2.89 log after treatment for 22 sec to 100^{o}C (Fig. 3.2B). Treatment of *Listeria monocytogenes* on alfalfa seeds at 8.1% moisture content for 16 sec to 85^{o}C did not significantly reduced populations (0.57 log CFU/g) without damaging the seed viability (Fig. 3.3B). The more effective treatment in killing *L. monocytogenes* was observed with

seeds at 6.4% moisture content treated for 12 sec to 82°C. This treatment significantly reduced the population by 0.89 log CFU/g (Fig 3.3A).

The relationships of dielectric heating exposure time, final temperature, germination rate and pathogenic populations reduction with different heating rate are shown in Figures 4-7. When the heating rate was 1.27°C/sec, the *Salmonella* population on the seeds with 3.2% moisture was reduced by 0.81 log CFU/g without inhibiting the germination of seeds after treatment for 69 sec to 113°C (Fig. 3.4A). The *Salmonella* population on the seeds with the same moisture content was reduced by 0.95 log CFU/g after treatment for 32.5 sec to 114°C when the heating rate was increased to 2.95°C/sec. Treatment of seeds with 7.9% moisture content at heating rates of 2.35 and 4°C/sec resulted in a similar reduction by about 0.9 log CFU/g of the population of *Salmonella* after being treated for 22 sec to 79°C and 20 sec to 98°C, respectively (Fig. 3.5). Treatment of seeds with 11.8% moisture content with heating rates of 1.59 and 2.42°C/sec did not reduce *Salmonella* population without damaging the germination rate (Fig. 3.6). Thus, treatment of seeds with 11.8% moisture content was the least effective in killing *Salmonella* on seeds even when the low heating rate was applied in this study. Treatment of seeds at 0.14°C/sec did not reduced significantly *L. monocytogenes* populations (0.67 log CFU/g) when treated for 360 sec to 73°C without damaging seed viability (Fig.3.7A). It is impractical to use exposure times greater than a few minutes. Reduction of *L. monocytogenes* population without damage to seed viability was not observed after treatment of 7.8% moisture content seeds at 6.07°C/sec due to the over heating at the shortest exposure (Fig. 3.7A). If heating rate is sufficiently high, the *L. monocytogenes* population is reduced but the germination rate is also reduced below

desired levels. Seed viability is more easily damaged when the moisture content of seeds is high.

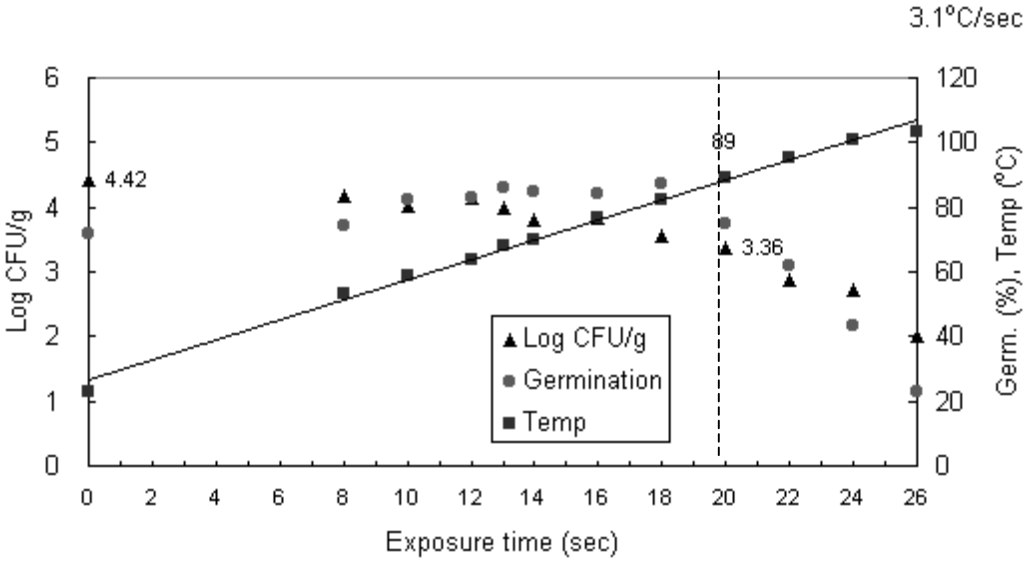
To date, with the exception of irradiation that reduced *S*almonella populations by more than 4 logs, no treatments have been reported to eradicate pathogenic bacteria on alfalfa seeds without adversely affecting the germination of seeds (Taormina and Beuchat 1999; Beuchat, 1997; Weissinger and Beuchat, 2000**;** Scouten and Beuchat, 2001). Dielectric heating also did not dramatically reduce the foodborne pathogens on alfalfa seeds without compromising the seeds viability. Increased germination percentage with a corresponding decrease in hard seed percentage was observed with dielectric heating. This phenomenon was reported in earlier studies (Nelson, 1976).

Sprouted seed samples evaluated for overall appearance and color were control (without inoculation and heating), inoculated seeds (5 and 8% moisture) without heating, and inoculated seeds (5 and 8% moisture) after heating. The overall appearance and color of treated and untreated sprouted seeds with either 5 or 8% moisture content were not significantly different ($P \leq 0.05$).

Overall, use of dielectric heating significantly reduced the pathogen populations without adversely affecting the germination percentage and vigor, but for desired reductions of pathogen populations, treatments to higher temperatures were required which reduced the desired seed viability. Without damaging the seeds viability, the most effective treatment in reducing the three pathogens were: seeds with 6.5 % moisture content, treatment for 20 sec to 89^{o}C (3^{o}C/sec heating rate) resulting in a 1.4 log population reduction of *E. coli* O17:H7; for the seeds with 6.5% moisture content treatment for 20 sec to 89^{o}C (3^{o}C/sec heating rate) resulted in a 1.06 log reduction of

Salmonella populations; and for seeds with 6.4% moisture content treatment for 12 sec to 82^{o}C (5^{o}C/sec heating rate) resulted in a 0.89 reduction of *L. monocytogenes*. Without damaging seed viability, the heat resistances of three pathogens are only slightly different. The most heat-resistant pathogen in this study was *L. monocytogenes*.

A.



B.

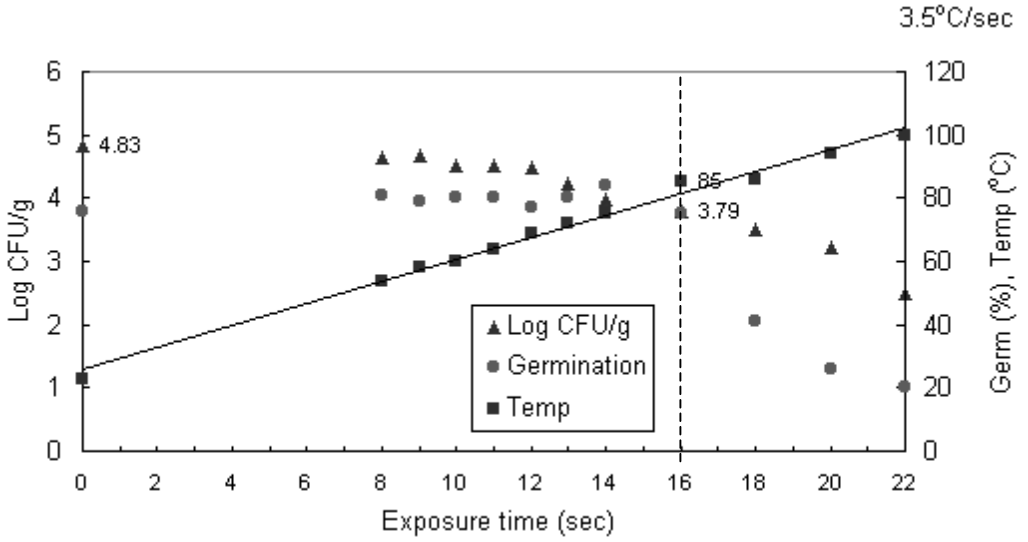
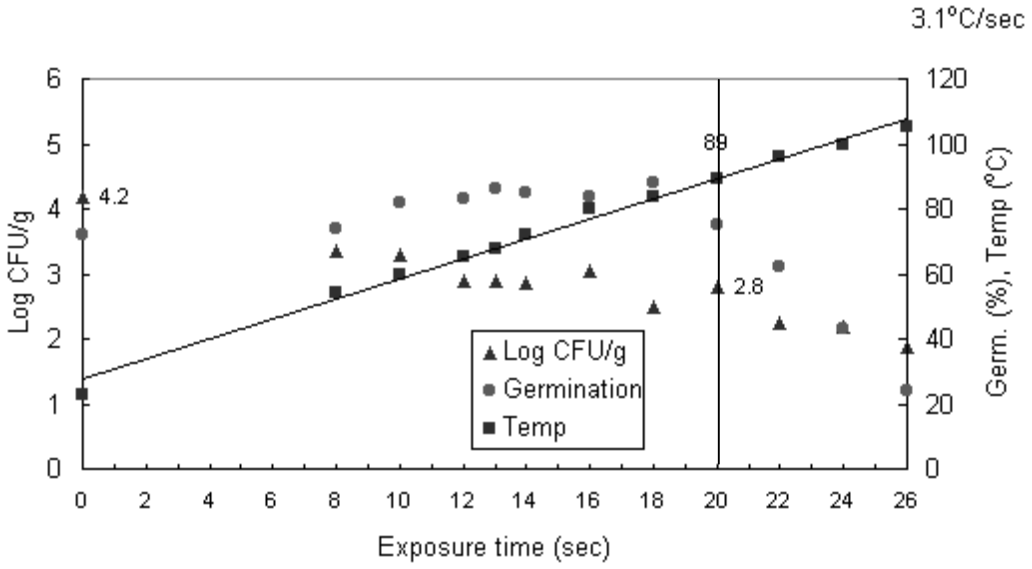


Fig. 3.1. Effect of dielectric heating on *Salmonella* populations on alfalfa seeds with either 6.5% (A) or 8.1% (B) moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 6.5% and 8.1% moisture were 3.1 and 3.5°C/sec, respectively.

A.



B.

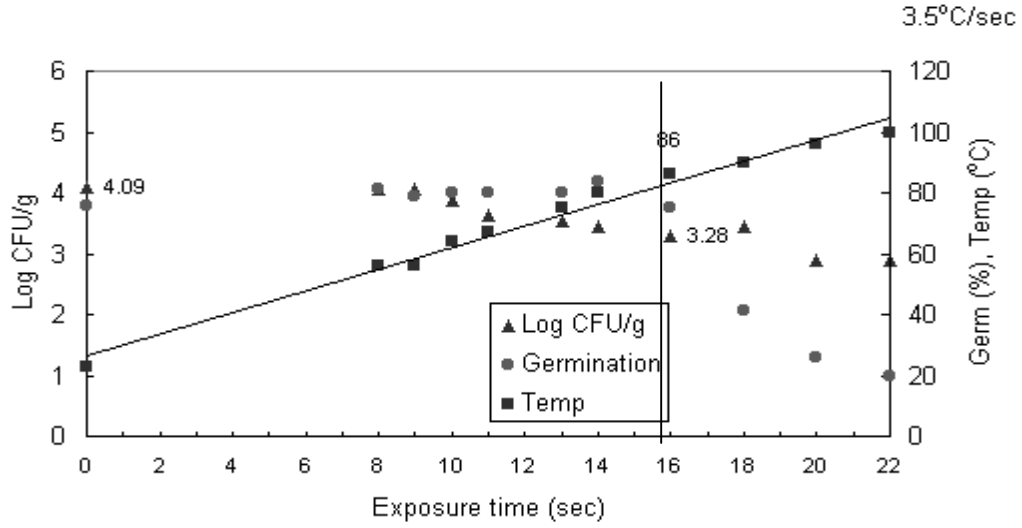
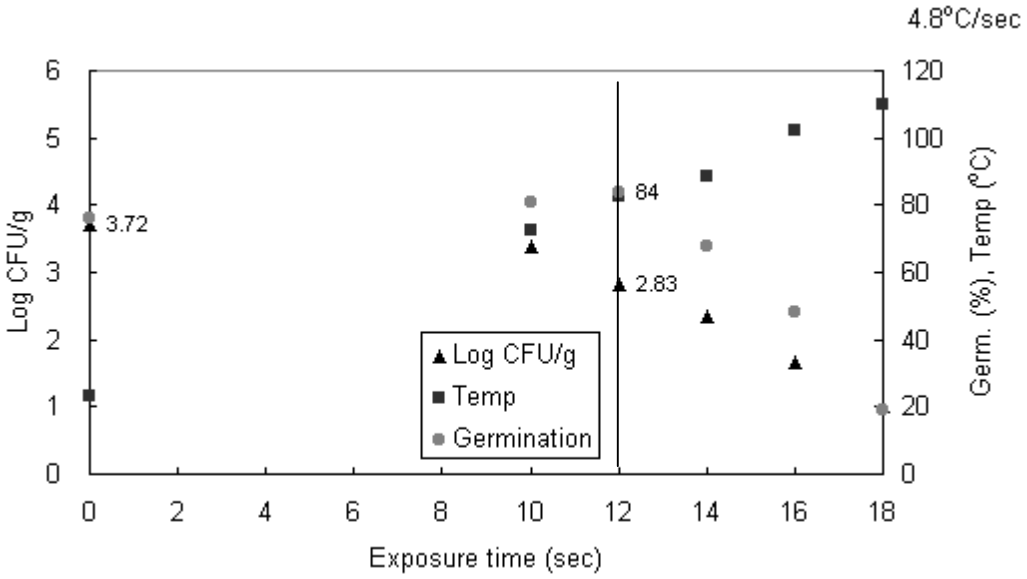


Fig. 3.2. Effect of dielectric heating on *E. coli* O157:H7 populations on alfalfa seeds with either 6.5% (A) or 8.1% (B) moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 6.5% and 8.1% moisture were 3.1 and 3.5°C/sec, respectively.

A.



B.

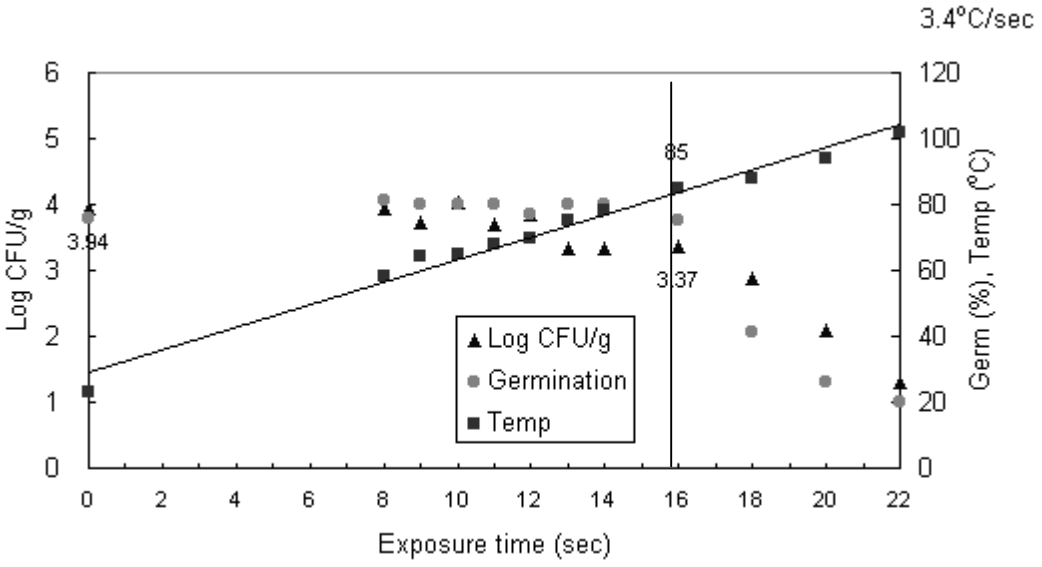
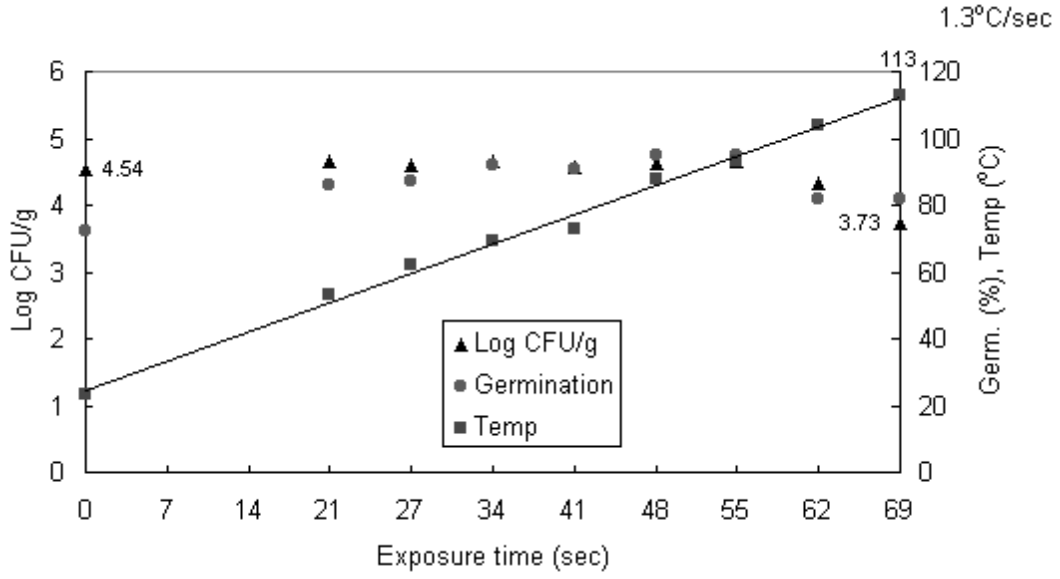


Fig. 3.3. Effect of dielectric heating on *L. monocytogenes* populations on alfalfa seeds with either 6.4% (A) or 8.1% (B) moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 6.4% and 8.1% moisture were 4.8 and 3.4°C/sec, respectively.

A.



B.

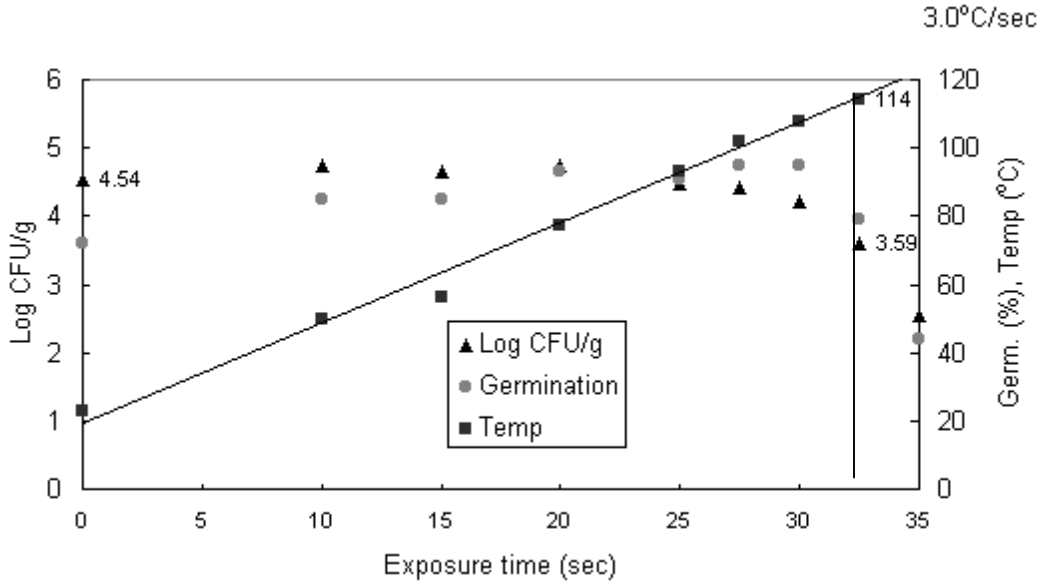
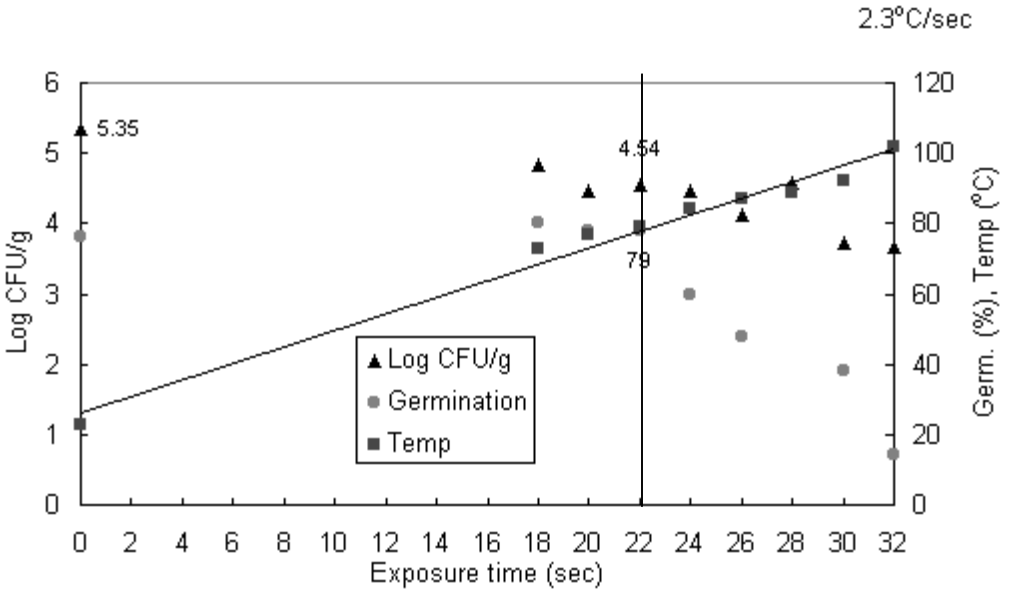


Fig. 3.4. Effect of dielectric heating on *Salmonella* populations on alfalfa seeds with either 3.2% moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 3.2% moisture were 1.3 and 3.0°C/sec.

A.



B.

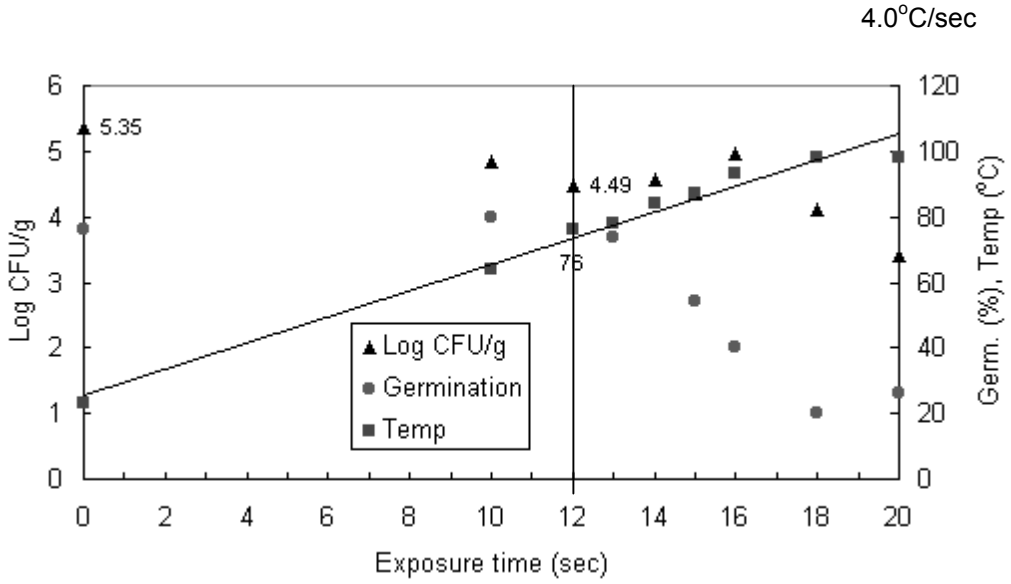
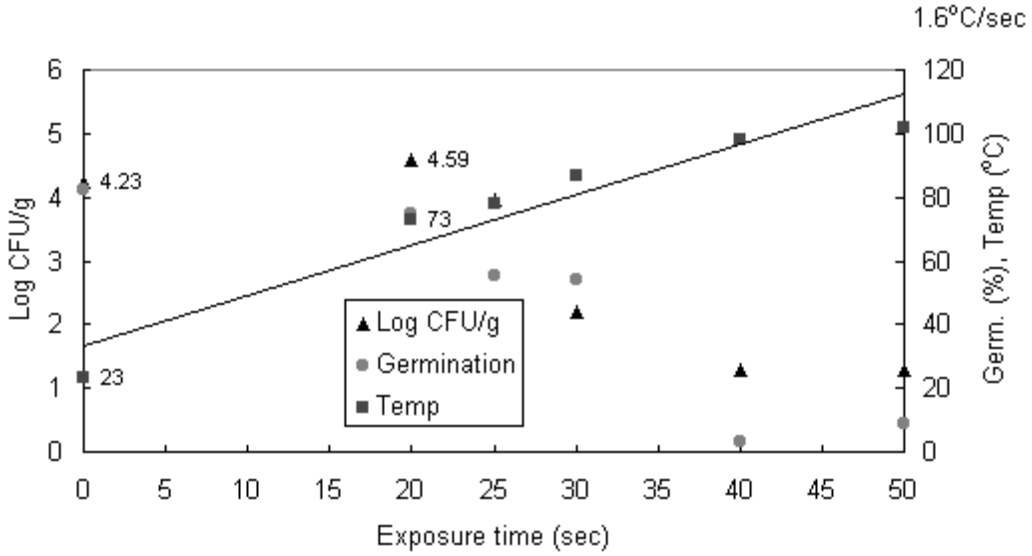


Fig. 3.5. Effect of dielectric heating on *Salmonella* populations on alfalfa seeds with either 7.9% moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 7.9% moisture were 2.3 and 4.0°C/sec.

A.



B.

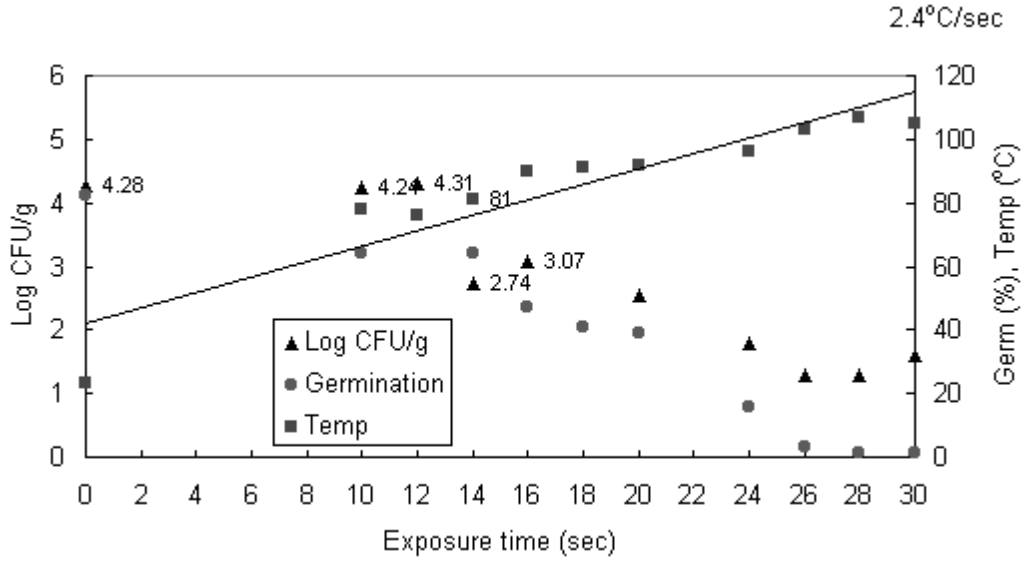
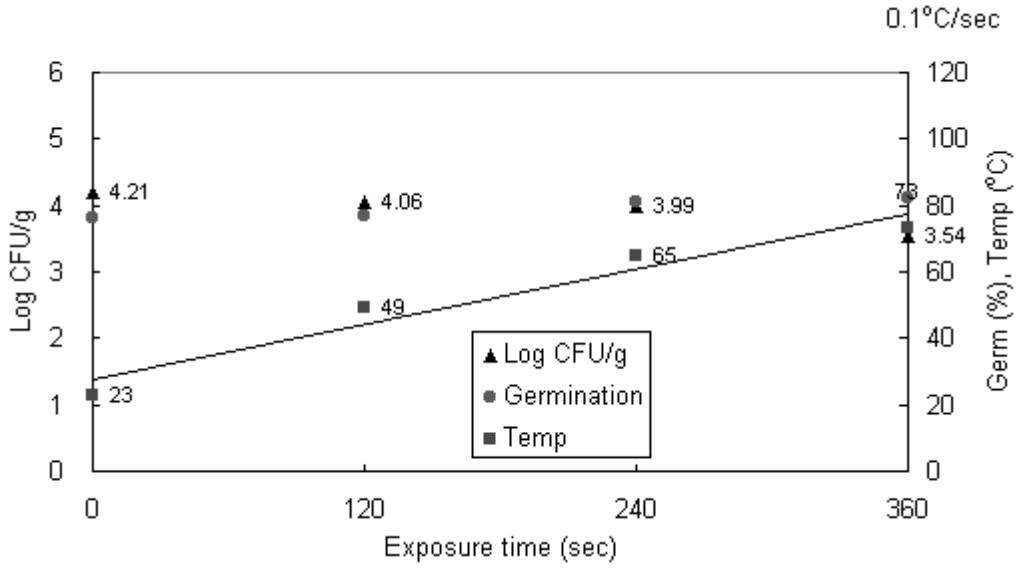


Fig. 3.6. Effect of dielectric heating on *Salmonella* populations on alfalfa seeds with either 11.8% moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 11.8% moisture were 1.6 and 2.4°C/sec.

A.



B.

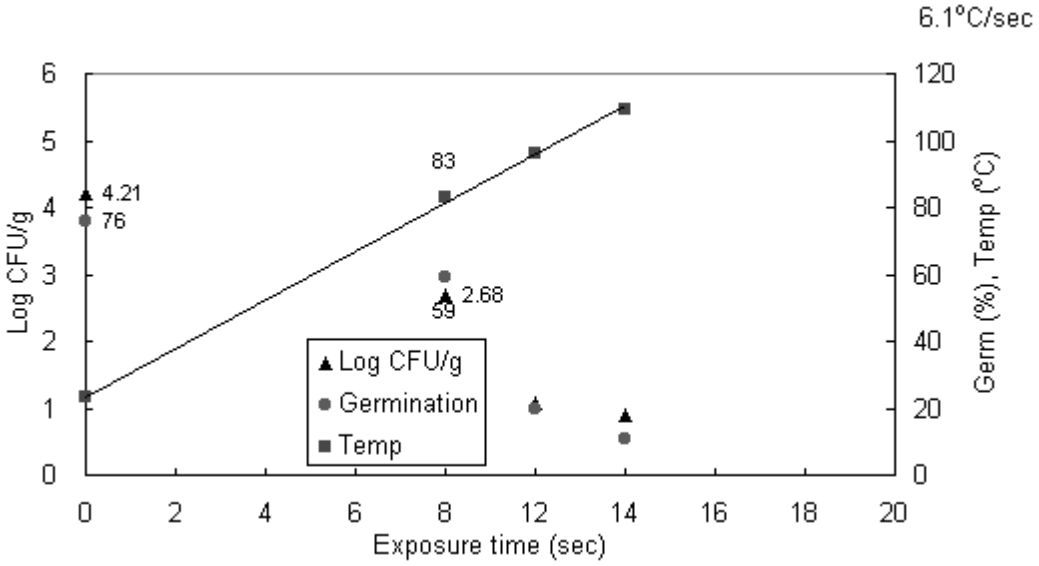


Fig. 3.7. Effect of dielectric heating on *L. monocytogenes* populations on alfalfa seeds with either 7.8% moisture content. Changes in seed germination rates and rate of heating are also noted. Heating rates for treated seeds with 7.8% moisture were 0.1 and 6.1°C/sec, respectively.

REFERENCE

Beuchat, L. R.1997. Comparison of chemical treatments to kill *Salmonella* on alfalfa seeds destined for sprout production. Int. J. Food Microbiol. 34:329-333.

Centers for Disease Control and Prevention. 1997. Outbreaks of *Escherichia coli* O157:H7 associated with eating alfalfa sprouts - Michigan and Virginia, June-July 1997. Morbid. Mortal. Weekly Rep. 46:741-744.

Cwiklinski, M. 2001. Effect of exposure to radio-frequency electric fields on *Fusarium graminerarum* in wheat seed. Tran. Am. Soc. Agric Eng. p. 01-6171.

Jaquette, C. B., L. R. Beuchat, and B. E. Mahon. 1996. Efficacy of chlorine and heat treatment in killing *Salmonella* Stanley inoculated onto alfalfa seeds and growth and survival of the pathogen during sprouting and storage. Appl. Environ. Microbiol. 62:2212-2215.

Lovett, J. 1989. *Listeria monocytogenes*, p. 283-310. *In*: Doyle, M. P. (ed.), Foodborne pathogens, Marcel Dekker, New York.

Lunden, A. O., R. C. Kinch. 1956. The effect of high temperature contact treatment on hard seeds of alfalfa. Agron. J. 359:151-153.

Mahon B.E., A. Ponka, W.N. Hall, K. Komatsu, S. E. Dietrich, A. Siitonen, G. Cage, P. S. Hayes, M. A. Lambert-Fair, N. H. Bean, P. M. Griffin, and L. Slutsker. 1997. An international outbreak of *Salmonella* infection caused by alfalfa sprouts grown from contaminated seeds. J. Infect. Dis. 175:876-882.

Morton, R. D. 2000. p.66. *In* Downes, F. P. and Ito, K. (ed.), Compendium of methods for the microbiological examination of foods. 4th ed., American Public Health Association. Washington DC, USA.

National Advisory Committee on Microbial Criteria for Foods. 1999. Microbial safety evaluation and recommendations on seed sprouts. Int. J. Food Microbiol. 52:123-153.

Nelson, S. O. 1976. Use of microwave and lower frequency RF energy for improving alfalfa seed germination. J. Microwave Power. 11(3):272-277.

Nelson S. O., L. E. Stetson, and W. W. Wolf. 1984. Long-term effects of RF dielectric heating on germination of alfalfa seed. Tran. ASAE 27(1):255-258.

Nelson, S. O., and E. R. Walker. 1961. Effects of radio-frequency electrical seed treatment. Agric. Eng. 42(12):688-691.

Nelson, S. O., and W. K. Whitney. 1960. Radio-frequency electric fields for stored grain insect control. Trans. ASAE 3(2): 133-137, 144.

Nelson, S. O., and W. W. Wolf. 1964. Reducing hard seed in alfalfa by radio frequency electrical seed treatment. Trans. ASAE 7(2):116-119, 122.

Nguyen-The, C., and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. Critical Reviews in Food Science and Nutrition. 34(4):371-401.

Ponka, A., Y., Anderson, A. Siitonen., B. deJong, M. Jahkola, O. Haikala, A. Kuhmonen, and P. Pakkala. 1995. Salmonella in alfalfa sprouts. Lancet 345:462-463.

Puohiniemi, R., T. Heisknen, and A. Siitonen. 1997. Molecular epidemiology of two international sprouts-borne *Salmonella* Outbreaks. J. Clinical Microbiol. 35:2847-2491.

Scouten A. J., and L. R. Beuchat. Combined effects of chemical, heat, and ultrasound, treatments to kill *Salmonella* and *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. (In press).

Taormina, P. J., L.R. Beuchat, and R. Slusker. 1999. Infections associated with eating seed sprouts: An international concern. Emerg. Infect. Dis. 5:629-634.

Taormina, P. J., and L.R. Beuchat. 1999. Comparison of chemical treatments to eliminated Enterohemorrhagic *Escherichia coli* O157:H7 on alfalfa seeds. J. Food Prot. 62:318-324.

Weissinger, W. R., and L. R. Beuchat. 2000. Comparison of aqueous chemical treatments to eliminate *Salmonella* on alfalfa seeds. J. Food. Prot. 63:1475-1482.

CHAPTER 4

SUMMARY AND CONCLUSION

Alfalfa seeds contaminated with human pathogenic bacteria have been associated with foodborne illness. Many studies have been done to investigate how to effectively kill human pathogenic bacteria on alfalfa seeds with various chemical solutions; however, to date, no treatments have reduced the pathogen populations completely. Other alternative methods for eradication of foodborne pathogens on the seeds are needed. Therefore, the two major objectives of the research investigated were:

1. To determine the efficacy of ultrasound treatment in combination with heat and chemical antimicrobial solutions in killing *Salmonella* on alfalfa seeds, and
2. To determine the efficacy of the application of dielectric heating in killing human pathogenic bacteria on alfalfa seeds and to investigate the sensory evaluation of sprouts from treated seeds.

Ultrasound treatment of alfalfa seeds at 38.5-40.5 kHz in combination with heat at 23 or 55^{o}C and chemical sanitizers slightly enhanced the effectiveness of chemical solutions in killing *Salmonella* without compromising the seed viability. Ultrasound treatment with 331 ppm Tsunami® 200 and 1200 ppm Sanova® were less effective in killing *Salmonella* on alfalfa seeds. Significant ($P \leq 0.05$) reductions of over 4.33 log CFU/g of *Salmonella* populations were reached with ultrasound in combination with 1% $Ca(OH)_2$ at 55^{o}C for 5 min, 1% $Ca(OH)_2$ plus 1% Tween 80 at 23 or 55^{o}C for 2 or 5 min, and 8% H_2O_2 at 23^{o}C for 5 min, 55^{o}C for 2 min. However, none of these treatments can completely eliminate the populations of *Salmonella* on seeds.

Dielectric heating caused significant ($P \leq 0.05$) reductions in the populations of *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes* on alfalfa seeds without adversely affecting the sensory attributes. Seed viability was easily damaged when the

moisture content of seeds was high, e.g., 11.8% moisture content. Therefore, the populations of pathogens were not reduced to desired levels before the germination rate was inhibited. With seeds at *ca.* 6.5% moisture content, dielectric heating reduced *E. coli O17:H7* populations by 1.40 log for 20 sec at 89^{o}C (3^{o}C/sec heating rate); *Salmonella* populations by 1.06 log for 20 sec at 89^{o}C (3^{o}C/sec heating rate); and *L. monocytogenes* by 0.89 log for 12 sec at 82^{o}C (5^{o}C/sec heating rate). An enhanced germination rate was observed during exposure with dielectric heating if exposures were not too long. In addition, sensory panelists determined the overall appearance and color of sprouts from treated seeds were not different from sprouts from untreated seeds.

This study found that physical treatments alone could reduce foodborne pathogens levels but not reduce their populations entirely without adversely affecting the germination percentage. Physical treatment in combination with exposure to chemical antimicrobial solutions enhanced the effectiveness of sanitization. However, none of these treatments in this study completely eliminated the pathogens. Combinations of an appropriate physical treatment with chemical solutions and sorting types of alfalfa seeds may improve sanitizing effectiveness on alfalfa seeds intended for sprouting.