Efficacy of Ultraviolet Light and Antimicrobials to Reduce *Listeria monocytogenes* in Chill Brines

By

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ABSTRACT

Chill brines used in ready-to-eat meat processing may be an important source of post-processing contamination by *Listeria monocytogenes*. The purpose of this study was to determine the efficacy of ultraviolet light (UV) in combination with antimicrobials to reduce *L. monocytogenes* in fresh and used chill brines. Three different antimicrobials were used in combination with UV; citric acid (CA, 0.2 and 0.5%), dimethyl dicarbonate (DMDC, 250 and 500 ppm), and hydrogen peroxide (HP, 2000 and 4000 ppm).

For fresh brine studies, brine (8.0% w/v NaCl) was prepared and inoculated with a cocktail of three *L. monocytogenes* strains (approximately 6 log CFU/mL). Brine was treated with UV alone, antimicrobials alone, and combination of UV and antimicrobials. Moreover, to observe the effect of treatment temperature and brine circulation through the UV system on survival of listeriae cells, inoculated brine was circulated through the system without any treatment that served as control for all the treatments. For UV treatment, inoculated brine solution was exposed to UV in an Ultraviolet Water Treatment Unit (Model: AMD 150B/1/2T D; Aquionics Inc., Peak output: 254 nm) fitted with an inline chiller to maintain brine temperature of -1°C. Samples were withdrawn at regular intervals for 120 minutes. When *L. monocytogenes* population was no longer detectable via direct plating on MOX, enrichment was performed and suspect colonies were confirmed using API-*Listeria*. For antimicrobial-only (i.e., no UV) treatments, a specific concentration of antimicrobial was added in inoculated brine and samples were taken for 120 minutes. For the brine that received combination of UV and

antimicrobial treatments, UV was turned on once a specific concentration of antimicrobial was added in inoculated brine and samples were withdrawn at regular intervals for 120 minutes.

When treated with UV alone, *L. monocytogenes* population decreased from approximately 6 log CFU/mL to below the detection limit (i.e., 1 log CFU/mL) in 15 minutes with the reduction rate of 0.87 log CFU/mL per minute. However, cells were detectable by enrichment through 120 minutes. The highest rate of decline (0.90 log CFU/mL per minute) was achieved by the combination of UV and 500 ppm DMDC (UV+500 ppm DMDC), which was not significantly different from the reduction rates of UV and UV+0.5% CA. UV+500 ppm DMDC reduced *L. monocytogenes* to the detection limit in 15 minutes and the organism was not detected by enrichment after 60 minutes. Though the reduction rate of UV+0.5% CA was not significantly lower than the rate of UV+500 ppm DMDC ($P>0.05$), the former treatment resulted in non-detectable levels more quickly (45 minutes) than the latter (60 minutes). Thus, based on enrichment studies UV+0.5% CA was the most effective treatment in reducing the population of *L. monocytogenes* in fresh brine. Moreover, when brine was treated with 0.5% CA alone the population decreased to below detection limit in 15 minutes with the rate significantly lower than UV+500 ppm DMDC and UV+0.5% CA ($P<0.05$). However, *L. monocytogenes* was not detectable by enrichment from 60 minutes. To summarize, through enrichment studies we observed that UV+0.5% CA, UV+500 DMDC, and 0.5% CA Control were more effective than other treatments in reducing the listeriae population to a non-detectable level.

Spent brine is recycled brine that was obtained from a frankfurter processor after its maximum usage. Results of spent brine studies showed that when brine was treated with UV+4000 ppm HP and UV+2000 ppm HP, *L. monocytogenes* population decreased to the detection limit in 45 minutes and was not detected by enrichment from 120 minutes. These

treatments were observed to be the most effective treatments with a reduction rate of 0.12 log CFU/mL per minute. The reduction rate of some other treatments such as, UV+250 and 500 ppm DMDC, UV+0.2% and 0.5% CA, and UV alone was not significantly different from UV+4000 and 2000 ppm HP. However, the population was detected through enrichment up to 120 minutes in all other treatments.

The results of these studies indicate that combinations of UV and antimicrobial may be more effective than either treatment alone (except 0.5% CA treatment) to process fresh chill brines. However, the antimicrobials and UV were less effective for controlling *L. monocytgoenes* in spent brine; presumably due to the presence of organic matter.

ATTRIBUTION

Author Priti Parikh is the major contributor and writer of the manuscripts in chapter four and chapter five of this dissertation. Co-authors Dr. Robert Williams, Ph.D., Food Science, University of Tennesse, 2001, Committee Chair, and Prof. Joseph Marcy, Ph.D., Food Science and Technology, North Carolina State University, 1980; Joseph Eifert, Ph.D., Food Science, Virginia Tech, 1994; Kumar Mallikarjunan, Ph.D., Biological Engineering, University of Guelph, 1993, Committee members, provided advice, supervision, funding, and laboratory support.

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DEDICATION

I dedicate this dissertation to my parents

Drs. Kishor and *Ranjan Shastri*, and

my wonderful husband, *Dr. Pratik Parikh*

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I joined the Department of Food Science and Technology (FST) at Virginia Tech in Spring 2005. It might not be a long time for me being in this wonderful department, but a lot of things have happened in this short time. I initially was enrolled in the Masters program at FST and then changed my status from M.S. to Ph.D. after a year. There are many people who helped me take that decision and I would always be grateful to all of them. So let me take this opportunity to thank everyone who directly or indirectly helped me fulfill my dream.

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CHAPTER: I

INTRODUCTION

Microbiological safety of foods and foodborne illness are complex issues since there are more than 200 known diseases that are transmitted through foods. Primary causative agents of foodborne illness are viruses, bacteria, parasites, microbial toxins, and prions. Table 1 lists some of the most commonly known foodborne pathogens.

Table 1. Most Commonly Recognized Foodborne Pathogens

Bacteria	Virus	Parasite	Molds
Listeria monocytogenes	Norovirus	*Giardia lamblia*	*Aspergillus* spp.
Salmonella spp.	Rotavirus	*Cryptosporidium parvum*	*Penicillium* spp.
Campylobacter spp.	Astrovirus	*Toxoplasma gondii*	*Fusarium* spp.
Escherichia coli O157:H7	Hepatitis A virus	*Cyclospora cayetanensis*	
Staphylococcus aureus		*Trichinella spiralis*	
Clostridium perfringens			

The symptoms of these foodborne illnesses range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes. Center for Disease Control and Prevention (CDC) has estimated approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths in the U.S. each year, out of which approximately 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths were due to known foodborne pathogens. Moreover, CDC recognized *Salmonella*, *Listeria monocytogenes*, and *Toxoplasma gondii* as leading causes of death since they were responsible for 1,500 deaths each year; i.e., more than 75% of deaths caused by known pathogens when they accounted for only approximately 11% of total cases of foodborne illness *(58)*.

It is, therefore, mandatory for food processors to implement pathogen reduction strategies accepted by regulatory agencies, such as U.S. Department of Agriculture (USDA) and Food and Drug Administrations (FDA), to ensure microbiological safety of foods. There are several technologies that have been in use in industry to process foods to increase their shelf life and improve microbial safety of foods.

Traditional Food Processing/Preservation Technologies

Foods are processed using various processing technologies to reduce or remove any potential pathogen or biological hazard that might be introduced while handling or processing. Foods can be processed with or without the application of heat. Traditional food processing technologies, such as pasteurization and heat sterilization, use heat/thermal treatment to kill or inactivate microbiological contaminant. Pasteurization is relatively a mild heat treatment technique in which food is heated to a temperature lower than 100°C *(32)*. This process is used to minimize potential health hazard through destruction of non-spore forming pathogenic micro-organisms. It also kills 99-99.9% spoilage micro-organisms and inactivates enzymes, in turn extending the shelf life of a product *(60)*. In contrast, heat sterilization is a severe heat treatment technique in which foods are heated at sufficiently high temperatures for prolonged time-periods to destroy microbial and enzyme activity. As a result, sterilized foods are shelf-stable with more than six months of shelf life *(32)*. A sterilized product may contain a viable spore that cannot grow due to environmental conditions, such as low pH, low water activity, etc., and still be commercially sterile *(60)*.

Even with a long history of use of heat in food processing, it has some limitations. Heat alters or destroys components of foods that are responsible for their individual flavor, color,

taste, or texture, and as a result they are perceived to have lower quality and value *(32)*. As consumers increasingly perceive fresh foods or minimally processed foods as healthier as compared to heat-treated foods, the industry is now developing alternative processing technologies. Alternative processing technologies have minimal process-induced changes in sensory and nutritional characteristics of the foods. Therefore, alternative processing technologies are now being used to effectively destroy any microbial threat, and to maintain the quality and storage-stability of foods *(1)*.

Alternative Processing Technologies

Alternative processing technology is also referred to as "non-thermal." These technologies include ultra violet light, high pressure processing, irradiation, pulse electric field, etc. Ultraviolet (UV) energy is a non-ionizing radiation that has germicidal properties at wavelength ranging from 200-280 nm. This UV range is also referred to as UV-C *(10, 50)*. Food treated with high pressure processing is exposed to a high hydrostatic pressure up to 1000 MPa for few minutes *(39)*. Gamma radiations and electron beams are commonly known as ionizing radiations that generate doses of 2-10 kGy *(30)*. Pulsed electric field treatment delivers pulses at high electric field intensity, 5-55 kV/cm, for a few milliseconds *(48)*.

Among all these technologies, UV-C radiation has the advantage that it does not produce chemical residues, by-products, and residual effect. Moreover, it is a simple dry and cold process *(5, 61)* that is inexpensive and requires very low maintenance as it does not need energy as a treatment medium *(43)*. The Food and Drug Administration has approved the use of UV treatment for pathogen reduction in water. The use of UV is a promising bactericidal alternative for other applications since it does not undesirably affect the color, flavor, odor, or taste of the

product *(100)*. Moreover, UV radiation does not produce undesirable by-products and is effective against a wide spectrum of microorganisms *(19)*. Thus, there is an increasing interest in using UV-C light for food disinfection due to its advantages over other treatments *(83)*. Since the UV has a high potential in disinfection of foods at a relatively low cost with several other advantages, we have considered using UV in this research. However, there a limitation of using UV-C that is its poor penetration ability. To overcome this UV light source must be placed as close as possible to the target in the processing system *(43)*.

It is worth noting that most of the alternative processing technologies achieve the equivalent of pasteurization, but not sterilization *(51)*. Therefore, to improve food safety antimicrobial agents have been used along with traditional and alternative food processing technologies.

Antimicrobial Agents

Antimicrobial agents are naturally occurring or artificially developed agents that have the potential of improving the microbial safety of foods *(24)*. Some of these substances are organic acids, hydrogen peroxide, dimethyl dicarbonate, nitrites, sodium chloride, sulfites, etc. Several research studies have shown that the use of antimicrobial agents can improve the efficacy of food processing technologies that use UV, pulse electric field, heat, or other antimicrobial agents. For example, Quicho (2005) illustrated that UV in combination with hydrogen peroxide or dimethyl dicarbonate is an effective treatment for producing a 5-log or greater reduction of *E. coli* O157:H7 in apple cider *(76)*. Combination treatments of 3 ppm ozone with 1% citric acid showed greater antimicrobial effect on indigenous microorganisms in enoki mushrooms than either 3 ppm ozone or 1% citric acid alone *(70)*. Pol et al. (2000) showed

that pulsed-electric field treatment enhances the bactericidal effect of nicin against *Bacillus cereus (74)*. Phillips and Duggan (2002) showed the synergistic effect of nicin with high temperature and various concentration of citric acid to reduce the population of *A. butzleri* in culture *(73)*. The addition of nisin in combination with sodium lactate significantly enhanced the effect of sodium lactate against *A. butzleri (72)*. This study also showed that when 500 UV/mL nisin was added in combination with lactic and citric acid then no viable cells were detected after 30 min incubation; whereas citric acid or lactic acid alone took 7.5 h and 30 h, respectively, to reach to non-detectable level. Such a combination of two or more treatments used to obtain a more effective method of food preservation is also referred to as *multiple hurdle technology (38)*.

This research, therefore, mainly focuses on the use of multiple hurdle technology that combines a non-thermal process, such as UV, with antimicrobials to improve the microbial safety of food.

CHAPTER II

PROBLEM STATEMENT

The Department of Health and Human Services in the United States has launched a comprehensive, nationwide health promotion and disease prevention agenda named "Healthy People 2010." The program objectives are designed to improve the health of all people in the first ten years of the 21st century. The primary focus areas of the initiative include improved food safety in the United States and a reduction in the incidence of foodborne diseases caused by *Listeria monocytogenes*, *Campylobacter*, *E. coli* O157:H7, and *Salmonella (62)*.

Over the last 25 years, listeriosis has been recognized as a major foodborne disease. Listeriosis is a foodborne disease caused by a Gram positive organism, *Listeria monocytogenes*. There are approximately 2000 cases of listeriosis reported every year in the United States, with approximately 500 fatalities *(60)*. Moreover, the economic burden of listeriosis has been estimated to be $255 million per year and associated food recalls were estimated to cost $15 million in 1985 through 1987 *(8)*. Even though the statistics show that the incidence of listeriosis has declined, outbreaks and contaminated product recalls continue to occur *(14)*. It is, therefore, very important to eliminate this pathogen from foods because of the high risk, fatality rate, and cost of listeriosis.

L. monocytogenes can be found in a wide variety of raw and processed foods. Milk and dairy products, various meats and meat products such as beef, pork, fermented sausages, fresh produce such as radishes, cabbage, seafood and fish products have all been associated with *Listeria* contamination. Foods such as soft cheeses, hot dogs and seafood have been implicated in several outbreaks of human listeriosis *(79)*. *L. monocytogenes* is a significant problem for the

food industries due to its ability to survive and grow under adverse conditions (e.g., low temperature, pH, water activity, etc.) that are not tolerated by other non-spore forming foodborne pathogens *(60)*. However, *Listeria* is not resistant to typical food processes such as cooking, pasteurization, or drying. So, it is primarily an environmental (i.e., food processing environmental) contaminant responsible for post-processing contamination of products.

Samelis and Metaxopoulos (1999) studied the occurrence and distribution of listeriae in a meat processing plant and determined the major sources and routes of contamination. They isolated *L. monocytogenes* and other *Listeria* spp. from 51% and 49% of frozen raw meat samples that were taken from several incoming lots. As a result, listeriae colonized at certain processing sites where raw materials were handled and stringent hygienic conditions were not applied *(82)*. In an extensive review, Tompkin (2002) noted that foods that have been involved in human listeriosis cases typically contain >1000 CFU/g or mL. Moreover, outbreak strains typically have become established in the food processing environment and as a result a lot of food gets contaminated frequently *(89)*. Consequently, *L. monocytogenes* is predominantly problematic in ready-to-eat foods; i.e., foods that are not going to be cooked by consumer.

Due to high risk of listeriosis and low infective dose, there is a "zero tolerance" policy for *L. monocytogenes* in ready-to-eat (RTE) foods in the U.S. Since this organism can grow at temperatures as low as 1°C, it poses a serious food safety hazard in refrigerated RTE products. Among RTE foods, meat and poultry products are the leading vehicles for human listeriosis *(47)*. RTE meat products, such as frankfurters that have received heat treatment followed by cooling in brine before packaging, may provide a more favorable environment for growth of *L. monocytogenes* because of the decreased competitive micro-flora and high salt tolerance of this organism *(27)*.

Frankfurter processing

Frankfurters are among the most important sausage products in the U.S. Generally, they are made from finely ground cured beef, pork, turkey and/or chicken that is called a frankfurter meat emulsion. The emulsion is pumped to a casing that is automatically twisted to form links. The links are then cooked by passing through hot water or steam and then hung for smoking, or smoking may precede the final cook *(75)*. During this process, frankfurters are heated to an internal temperature of 155°F to set the emulsion and produce the desired color *(81)*. The inability of *L. monocytogenes* to withstand such a high temperature reduces the risk of its survival in frankfurters when contamination occurs in the raw ingredients. However, if the processing environment is contaminated by *Listeria*, then frankfurters can become contaminated after processing and provide an adequate pH, water activity, and nutrients for the growth of this pathogen. In the past, frankfurters have been linked to several outbreaks. It has also been noticed that sometimes frankfurters are eaten without reheating to a sufficient temperature *(16)*.

There are several phases of frankfurter processing where contamination with *Listeria* is more likely. For example, cellulose casing that is used to form the shape of frankfurters is mechanically peeled off with a knife after cooking, and cooked frankfurters are transferred by conveyors. So if the knife, conveyor, or any of the packaging materials are contaminated with *Listeria*, then cooked RTE frankfurters may be contaminated with this organism *(81, 90)*.

Another potential point in the process where *L. monocytogenes* contamination may occur is during product cooling. Chill brine is used to quickly cool thermally processed RTE products such as frankfurters, deli meats, fish, cheese, etc., or raw products. This treatment provides two microbiological benefits. First, it assures adequate shelf life by preventing growth of spoilage microorganisms. Second, it helps improve microbial safety of foods by preventing growth of

pathogenic organisms that may survive thermal processing *(59)*. However, products often introduce heat and nutrients to brine allowing *L. monocytogenes* and other organisms to survive, grow, and spread in brines. It has been studied that contamination of frankfurters by *L. monocytogenes* can occur during the cooling step when the frankfurters are sprayed with chilled salt brines before packaging *(59)*. Chill brine is often recycled, which helps reduce the costs of processors. It is, therefore, very essential to maintain the microbial safety of chill brine especially when it is recycled. The USDA has set limits for maximum usage of chill brines based on the type of casing and salinity and temperature of the brine. Under certain conditions brines may be used for up to four weeks. Table 2 summarizes the required temperature and salt concentration for recycling of chill brine used for heat treated products *(91)*. USDA also recommends that food processing industries or establishments perform the ongoing monitoring of the recycled brine solution, as illustrated in Tables 3 and 4, that is used for cooked and raw or heat-treated products *(91)*.

Table 2: USDA regulations for recycled chill brines *(91)* (USDA)

Maintenance Conditions for Brine Used for Heat Treated Products		
Duration of Use	*Minimum salt concentration (%)*	*Maximum temperature*
One production shift	None	None
Up to 24 hours	5	40°F (4.4°C)
Up to 1 week	9	28°F (-2.2°C)
Up to 4 weeks	20	10°F (-12.2°C)

Table 3: Monitoring of the Recycled Brine Solution Used for Cooked Products (USDA)

Analysis	**Frequency**	**Action level**
Total Plate Count	Daily	> 2500 CFU/mL
Total Coliform	Weekly	Positive
Fecal Coliform	Weekly	Positive

Table 4: Monitoring of the Recycled Brine Solution Used for Raw or Heat-Treated, Raw Not Fully Cooked Products (USDA)

Analysis	Frequency	Action level
Total Plate Count	Daily	> 5000 CFU/mL
Total Coliform	Weekly	>10 CFU/mL
Fecal Coliform	Weekly	Positive

L. monocytogenes can survive and grow at high salt concentration (around 10-12%) and low temperatures (up to 0°C) and thus chill brine can harbor this organism once contaminated. Several studies have shown that contamination of RTE products increases after brining and the most contaminated sites of the processing plant are the brining and post-brining areas *(4, 42)*. Thus, it is very important to prevent *L. monocytogenes* survival in brines especially when they are recycled.

Previous studies have shown that UV is effective for reducing the population of various microorganisms in brine, water and certain other foods *(9, 19, 37, 49, 56, 80, 94, 95)*. Antimicrobial agents such as citric acid, hydrogen peroxide, and dimethyl dicarbonate are effective under certain conditions. Therefore, this study is designed to determine the efficacy of UV and chemical preservatives for the reduction of *L. monocytogenes* in chill brines.

CHAPTER: III

LITERATURE REVIEW

A. *Listeria monocytogenes*

Characteristics of the Organism

The genus *Listeria* comprises six recognized species including, *Listeria monocytogenes, Listeria ivanovii*, *Listeria innocua*, *Listeria seeligeri*, *Listeria welshimeri*, and *Listeria grayi*. Although all of these species are pshychrotrophic and widely spread in the environment, only *L. monocytogenes*, and *L. ivanovii* are known to be pathogenic. *L. monocytogenes* is a human pathogen whereas *L. ivanovii* is primarily an animal pathogen. However, the ability to lyse red blood cells differentiates *L. monocytogenes* from non-pathogenic *Listeria* spp.

L. monocytogenes is a Gram-positive, motile, non-spore forming, rod shaped bacterium. It can multiply at temperatures approximately 0°C and it can survive even at freezing temperature. It is micro-aerophilic and it can grow in the presence of 10-12% sodium chloride. Certain strains may grow at water activity as low as 0.90. Heat resistance of *L. monocytogenes* increases as the water activity of the food decreases. Therefore, it creates problems for food manufacturers who use low water activity and heat treatments to maintain safety *(60)*.

Characteristics of the Disease

As mentioned earlier, the disease caused by *L. monocytogenes* is called listeriosis. In the US, approximately 2,000 people become seriously ill with listeriosis each year and around 500 of these people die. *L. monocytogenes* causes disease in certain high-risk groups including, pregnant women, neonates, the elderly, and immunocompromised adults. Occasionally, listeriosis occurs in healthy adults. A person infected with *L. monocytogenes* usually shows flu-like symptoms initially; i.e., fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. In non-pregnant adults, *L. monocytogenes* can also enter in the bloodstream and cause septicemia. If the infection spreads to the nervous system, then it can cause meningitis and meningoencephalitis. The mortality rate of *Listeria* infection is 20-25%. Infected pregnant women generally only display mild flu-like symptoms. However, infection during pregnancy can lead to miscarriage, premature delivery, infection of the newborn with serious long-term consequences, or even stillbirth *(31, 60)*.

Foodborne outbreaks

The first outbreak of *Listeria* was documented in 1981 in Canada. The source of this outbreak was coleslaw. Subsequently, many of the *Listeria* foodborne outbreaks that have been reported in the US and other countries have been linked to deli turkey meat, coleslaw, Mexican-style cheese, and soft cheese *(60)*. Moreover, *L. monocytogenes* has triggered a number of recalls for poultry and meat products from 1994-2001 (Figure 1).

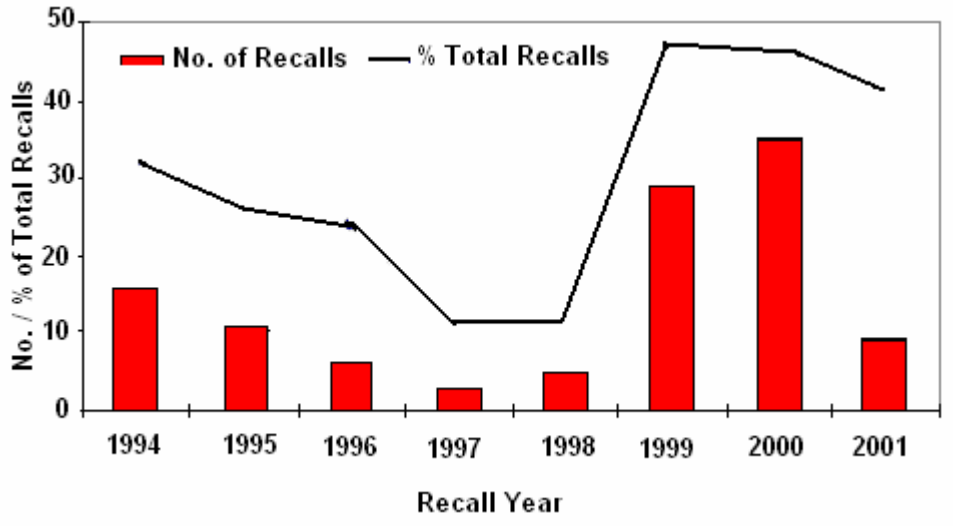

Figure 1: Meat and poultry recalls due to *L. monocytogenes* from 1994-2001 (data from *(35)*)

There were 35 *Listeria* recalls have been reported to USDA FSIS (Food Safety and Inspection Service) from 2005 to 2006 *(92)*. These recalls were mainly for various fully cooked chicken products, frozen egg rolls, ready-to-eat meat products, and sausage products. In October 2002, a multistate outbreak of *L. monocytogenes* infection with 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages was reported in eight northeastern states of the US. As a result of this outbreak, Pilgrim's Pride Foods (PA) recalled 27.4 million lbs. of fresh and frozen ready-to-eat turkey and chicken products (considered as the largest poultry recall in history) and Jack Lambersky Poultry Company (NJ) recalled around 4.2 million lbs. of fresh and frozen, ready-to-eat poultry products *(13)*. During October 2000 – January 2001, an outbreak of listeriosis associated with homemade Mexican-style cheese was reported in North Carolina. In this outbreak twelve cases were identified including 10 pregnant women and resulted in five stillbirths, three premature deliveries, and two infected newborns *(18)*. There was also a ten state outbreak of listeriosis between May and November reported in 2000. Eight perinatal and 21 nonperinatal cases were reported. From early August 1998 to January 1999, at least 50 illnesses

caused by *L. monocytogenes* (serotype 4b) were reported by 11 states *(17)*. In 1994 listeriosis outbreak attributed to contaminated chocolate milk was reported among people who attended a picnic in Illinois. In this outbreak forty-five people were found who had symptoms that met the case definition for illness due to *L. monocytogenes*, and cultures of stool from 11 people yielded the organism and four persons were hospitalized. The most common symptoms were diarrhea (present in 79 percent of the cases) and fever (72 percent). The disease causing strain was isolated from a valve connected to the chocolate milk filler and a drain under the filler *(23)*. In 1985, contaminated Mexican-style cheese caused listeriosis outbreak in California. There were 142 cases reported in eight months *(15)*.

Susceptibility to Various Environmental Conditions

1. *Temperature*

The optimum temperature for the growth of *L. monocytogenes* is approximately 30°C, whereas it can grow at a temperature ranged from 0°C to 45°C. Temperatures higher than 50°C inactivate this organism and thus, pasteurization or thermal processing of the foods help eliminate this organism *(60)*.

L. monocytogenes can survive even at freezing temperature and thus creates problems with frozen foods, although survival and injury during frozen storage depend on the substrate and the rate of freezing *(60)*. Therefore, it is necessary to control storage temperature to minimize the growth of this pathogen. Glass and Doyle (1989) showed that *L. monocytogenes* can survive on various types of meat products, such as bologna, wieners, sliced chicken and turkey, and sausages, which are stored at 4.4°C. The growth rate however depends on the type of product and the highest proliferation was found on poultry products *(40)*. Another study

illustrated that 65.6% of Canadian retail wieners inoculated with *L. monocytogenes* supported the growth of this pathogen when stored under vacuum at 5°C for up to 28 days *(57)*. However, at lower temperature the replication of bacteria slows down. Papageorgiou and others reported generation times of *L. monocytogenes* ranged from 16.2 to 20.2 h in whey cheeses that were significantly longer than those observed at 12°C, which ranged from 5.1 to 5.8 h. At 22°C, the generation time ranged from 1.7 to 2.7 h *(68)*.

2. *pH*

L. moncytogenes encounters a low pH environment in acidic foods, during gastric passage, and in the phagosome of the macrophage *(38)*. Like temperature, *L. monocytogenes* can grow at a wide range of pH; typically reported as 5.6 – 9.6. Moreover, recent studies show that the organism can initiate growth in laboratory media at pH values as low as 4.4 *(60)*. This pathogen responds to and survives at so low pH by utilizing a number of stress adaptation mechanisms. Exposure of *L. monocytogenes* to mild acidic pH of 5.5 causes the acid tolerance response (ATR), wherein the cells are resistant to severe acidic conditions *(65)*. It has been observed that the minimum pH supporting the growth of *L. monocytogenes* is dependent upon the temperature of incubation, available nutrients, moisture content, and composition of the food product *(47)*. Parish and Higgins (1989) studied the survival of *L. monocytogenes* strains in low pH model broth system. They observed growth in the broth at pH 4.5 and higher for all strains during incubation at 30°C, but not at pH 4.0 or lower *(69)*.

3. *Salt Concentration*

The use of salt to lower the water activity is one of the methods of food preservation used by food industries. However, the ability of this organism to adapt and survive in high salt concentrations makes it difficult to control in foods *(38)*. *L. monocytogens* can grow in the presence of 10-12% sodium chloride, and it grows to a high population in moderate salt concentrations (6.5%). This organism can survive for longer periods at higher salt concentration. And, its survival is significantly increased by lowering the temperature *(60)*. Hudson (1992) studied the efficacy of high sodium chloride concentrations to destroy the population of *L. monocytogenes* and showed that the pathogen would grow at refrigeration temperature when salt concentration is relatively low (6%). Population could be reduced in the presence of high salt concentration (26%), but not in practical time periods for use in the food industry *(46)*.

4. *Water Activity*

The optimum water activity for the growth of *L. monocytogenes* is ≥ 0.97. For most strains of *L. monocytogenes*, the minimum water activity for the growth is 0.90. However, the organism can survive for longer period of time at water activity values as low as 0.83. Since thermal resistance of the organism increases with the decreased water activity, great care should be taken by manufactures that rely on the low water activity and thermal treatment for the preservation of food products *(60)*.

Tapia de Daza et al. studied the effect of solute and temperature on water activity for growth of *L. monocytogenes* strain Scott A and Brie 1. They used glycerol, NaCl, and sucrose to reduce the water activity of Tryptic Soy Broth (TSB) to 0.90. They observed that both the

strains grew well at 30°C in glycerol supplemented TSB, but not in NaCl and sucrose supplemented TSB. Also, increased effect of all the supplements was observed at 4°C. Thus, the type of solute and osmotic conditions created by the solute affect the ability of *L. monocytogenes* to grow *(88)*.

Contamination of Ready-to-Eat (RTE) Products

Some RTE food products, such as frankfurters, delicatessen meat and poultry products, and soft cheeses, pose high risk of listeriosis especially for susceptible population. Since 20% of the refrigerators have temperatures of more than 50°F, it does not ensure the safety of RTE products. Moreover, *L. monocytogenes* grow well at refrigeration temperature so this provides increased risk of listeriosis from RTE foods *(60)*. Therefore, it is very important to prevent any contamination of RTE products with this organism.

Meat and Poultry Products

Growth of *L. monocytogenes* in meat and poultry products depends on the type of meat, pH of the product, and the presence of other bacteria. *L. monocytogenes* grows better in poultry than in other meats. It can grow rapidly in meat products, including vacuum-packed beef, at pH values near 6.0 and little or no growth at pH values around 5.0. Moreover, it is very difficult to remove or kill this organism once it is attached to the surface of raw meats *(60)*. Therefore great care should be taken while processing meat and poultry products. There are two primary modes of contamination of products with *L. monocytogenes*, through infected animal or bird and post-processing contamination.

Contamination of animal muscle tissue by *L. monocytogenes* can be caused in or on the animal before slaughter. This pathogen concentrates and multiplies in kidney, lymph nodes,

liver, and spleen *(60)*. Therefore, organ meat may get contaminated with listeriae cells if the animal is infected. However, the pathogen is primarily a post-processing contaminant. Therefore, it is possible to get contaminated finished poultry/meat products even though the birds or animals were not infected with the organism. *L. monocytogenes* can enter in the food processing plant since it is ubiquitous in nature, and contaminate the product through many routes. Once established in the food-processing environment, this pathogen is very difficult to remove and it becomes a source of recontamination of processed products. Lin et al. (2006) studied the cross-contamination between processing equipment and deli meats by *L. monocytogenes*. They showed that this organism can be transferred from a contaminated slicer onto meats and can survive or grow better on uncured oven-roasted turkey than on salami or bologna with preservatives *(54)*.

Another major source of post-processing contamination of meat products is 'brine.' Brine is a recycled medium that is commonly used to cool cooked product and to add flavors. Since, *L. monocytogenes* can grow at high salt concentration and low temperature; it can survive in brines and the brine can be an important source of contamination of finished products. Moreover, if the product is ready-to-eat, then the contamination can result in an outbreak or recall. However, USDA has some regulations on reuse of brines as discussed before (Table 1). Larson et al. reported the survival of *L. monocytogenes* in commercial cheese brines (5.6-24.7% NaCl) collected from cheese factories in Wisconsin and northern Illinois at 4 and 12°C *(53)*. Greer et al. (2004) showed that recycled brines can harbor large population of spoilage bacteria and *L. monocytogenes* and are an important source of contamination of moisture-enhanced pork *(42)*. Being able to control *L. monocytogenes* in chill brines may further extend their shelf-life, dramatically reducing the cost of water, salt, brine disposal, and time consumed for replacing

batches of brine, without fear of increased pathogen risk (Ye et al., 2001). Gailey and others (2003) studied the survival of *L. monocytogenes* in a simulated recirculating brine chiller system and showed that very few cells were destroyed during the process. However, they also observed the dilution effect during the study that indicated that if the system is regularly cleaned and sanitized, then brine chiller system poses little danger of postcooking contamination *(36)*.

Dairy Products

Unpasteurized or raw milk is a source of *L. monocytogenes*. A listeriosis outbreak in 1985 in California was associated with Mexican-style cheese that was made with unpasteurized milk *(15)*. Since high temperature kills this pathogen, pasteurization is used to process milk. However, *L. monocytogenes* grows more rapidly in pasteurized milk than in raw milk at 7°C and therefore post-process contamination is a major concern. Temperature abuse can then increase listeria growth *(60)*. In an outbreak associated with contaminated chocolate milk in Illinois, listeria population increased to 10^9 CFU/mL. This gave the milk an off flavor, but people still drank it *(23)*.

Among dairy products, *L. monocytogenes* has also been associated with a variety of cheeses. It can survive cheese manufacturing and ripening because of its resistance to low temperature and high salt concentration. This organism concentrates in cheese curd during manufacturing. However, the behavior of listeriae in the curd is influenced by the type of cheese. For example, "during cheese ripening, *L. monocytogenes* can grow in Camembert cheese, die gradually in Cheddar or Colby cheeses, or decrease rapidly during early ripening and then stabilize in blue cheese" *(60)*.

Seafood

L. monocytogenes has been isolated from fresh, frozen, and processed seafood products, such as crustaceans, molluscan shellfish, and finfish *(60)*. Gombas et al. studied the prevalence of *L. monocytogenes* in RTE foods and observed that prevalence rate of seafood salads (4.7%) and smoked seafood (4.3%) was highest among all the RTE products tested. Higher prevalence rate, such as 7.3% *(64)* and 79% *(28)* was found in the U.S. for smoked fish that were obtained from processing plants. Seafood that may pose high risk of listeriosis include, mollusks (such as mussels, clams, and oysters), raw fish, lightly preserved fish products (such as salted, marinated, fermented, and cold-smoked fish), and mildly heat-processed fish products and crustaceans *(60)*.

B. Ultraviolet radiation

Effect of UV on microorganisms

UV processing includes the use of radiation from the ultraviolet region of the electromagnetic spectrum to kill microorganisms. The wavelength for UV ranges from 100-400 nm (Figure 2).

This range is again divided into UV-A (from 315-400 nm), UV-B (from 280-315 nm), UV-C (from 200-280 nm), and vacuum UV range. In general, the wavelength between 220 and 300 nm is considered germicidal against wide variety of microorganisms such as bacteria, virus, protozoa, molds, yeast, and algae *(10, 61, 85)*. However, the highest germicidal effect is obtained between 250 to 270 nm and it decreases as the wavelength increases *(5)*. Therefore, UVC at wavelength of 254 nm, which is generated by low pressure mercury lamps, is used for disinfection of surfaces, water, and foods. UV light has different penetration capacity in

different physical media. Therefore, bacteria suspended in air are more sensitive to UV-C light than in liquid *(10)*.

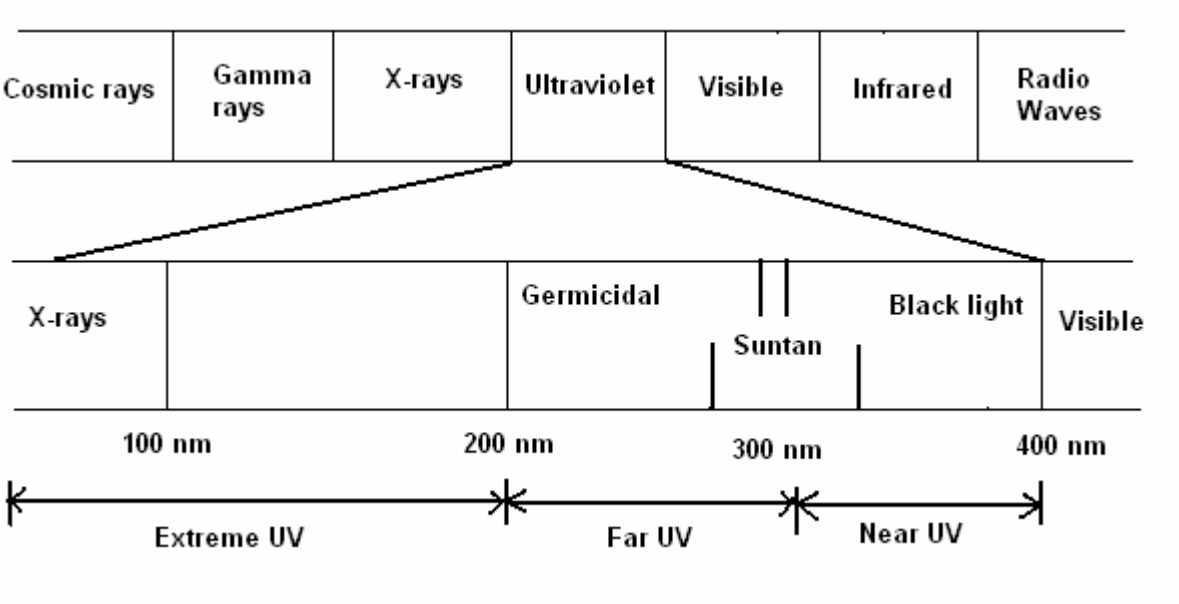

Figure 2: Electromagnetic Spectrum *(43)*

UV-C inactivates organisms mainly by DNA damage. DNA of microorganisms absorbs the UVC that causes cross-linking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand. And thus, DNA transcription and replication cease, leading to cell death *(60, 93)*. In addition, UV radiation cross-links aromatic amino acids at their carbon–carbon double bonds. The resulting denaturation of proteins contributes to membrane depolarization and abnormal ionic flow *(51)*. UV inactivation of microorganisms is mainly dependent on the UV dose and not the intensity of the light. Thus, low UV radiation intensity for long exposure time would give the similar effect as high intensity for a short time *(61)*. The UV dose is usually measured in 'mJ/cm^2' or 'J/m^2.' Generally, the UV dose required to inactivate viruses and molds is much higher than for bacteria. However, resistance of bacteria varies with

species and it also depends on their physical state (e.g., whether they are in the form of spores or vegetative cells, and age). Spores are more resistant to UV than vegetative cells. Moreover, vegetative cells are more resistant to UV just prior to active cell division, during lag phase. It has also been found that microbial resistance to UV radiation increases in absence of oxygen *(60, 93)*.

Efficacy and Applications

As mentioned earlier, the major limitation of using UV for foods is its inability to penetrate. UV-C cannot penetrate deeply into solid foods rather it acts only on the surface and has limited ability to penetrate in liquids. The penetration depth of UV-C in liquid media is very short with the exception of a clear water *(84)*. Therefore, to eliminate the same number of organisms the required UV dose is different in agar medium and foods *(87)*. The penetration of UV light into juices is about 1 mm for the absorption of 90% of the light and so a turbulent flow during liquid food processing is recommended *(44)*. The penetration ability of UV-C light depends on the type of liquid, soluble solids and suspended matter present in the liquid, and UV-C absorption capacity of the liquid. Large suspended particles may also block the incidence of light on the microbial load *(10, 84)*.

UV light has been used to inactivate various microorganisms in different foods. Exposure of shell eggs to UV light (254 nm) at 4350 μW/cm2 for 15 min reduced the aerobic microbial population by 3 log units *(50)*. Moreover, studies have shown that UV exposure does not negatively affect the quality of the product *(55)*. UV has been used as an alternative to chlorine to disinfect drinking water and has shown effectiveness against a variety of microorganisms *(19)*. It has also been used to disinfect air and surfaces in hospitals and research laboratories where aseptic environments are required. Moreover, in recent years, UV has been

used to treat packaging materials for aseptic packaging and to process juices *(93)*. Warriner and others (2000) demonstrated that UV inactivates the spores of *Bacillus subtilis* on packaging surfaces. A number of studies have been done on use of UV to process various food products *(95)*. Rodriguez and Yousef (2005) inactivated *Salmonella enterica* serovar Enteritidis present on shell eggs by UV radiation and ozone *(80)*. Studies by Marquenie and others (2002) showed that fungal growth on strawberries was significantly retarded using UVC dose of 0.05 J/cm^2 and higher *(56)*. Wright and others examined the efficacy of UV light (254 nm) for reducing the population of *E. coli* O157:H7 in unpasteurized cider. They observed that UV was effective in reduction of this pathogen, but the dosages used in this study were not adequate to achieve the required 5-log reduction *(97)*. UV-C (253.7 nm) has also been effective in inactivating certain foodborne pathogens such as *Salmonella* spp. and *E. coli* O157:H7 on fresh produce and fruits *(98)* and on agar surfaces *(99)* at different dosage level. Harrington and Hills (1968) obtained 2.67 log reduction in total count by processing apple cider with UV and observed a good quality shelf life during 35 days at 2.2°C *(45)*. When a thin film of orange juice was treated with UV system at 214.2 W/m^2, the orange juice doubled the shelf life without changes in color and taste *(29)*.

UV Inactivation of *Listeria monocytogenes*

Very little research has addressed inactivation of *L. monocytogenes* by UV. Ozer and Demirci (2006) studied the efficacy of pulsed UV-light to inactivate *L. monocytogenes* and *E. coli* O157:H7 on raw salmon fillets. They demonstrated that about one log reduction of *E. coli* O157:H7 or *L. monocytogenes* could be achieved with 60 second treatment at 8 cm distance without affecting the quality of the product *(66)*. Guerrero-Beltran and Barbosa-Canovas (2005) studied the efficacy of UV in reducing the population of *Saccharomyces cerevisiae*, *E. coli*, and

L. monocytogenes in apple juice. They used double tube UV disinfection treatment in this study. They found that *S. cerevisiae* was more resistant to the UV light treatment because the *Duv* values were higher than that for *L. innocua* or *E. coli*. The log reduction obtained in this study after 30 min of UV treatment was 1.34 ± 0.35, 4.29 ± 2.34 and 5.10 ± 1.12 for *S. cerevisiae, L.innocua* and *E. coli,* respectively, at the highest flow rate (0.548 L/min). High microbial reduction was also observed when a mixture of microorganisms was inoculated instead of inoculating separately *(44)*. Gailunas et al. (2003) showed that UV reduced the number of *L. monocytogenes* by around 4.6 and 4.5 log in 7.9% w/w and 13.2% w/w brine, respectively. Moreover, the temperature of the brine was increased by an average of 15.5°C in this study where the initial average temperature of the brines was 10.3°C *(37)*. A study by Kim and others (2002) showed that UV irradiation was effective in reducing the number of selected pathogens (e.g., *L. monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7) in peptone water and on the surface of stainless steel chip. However, UV did not significantly reduce pathogens inoculated on chicken meat surfaces with or without skin. Another study by Bintsis et al. (2000) showed that long-wave UV light (UVA~365 nm) with the intensity of 45 W/m^2 along with psoralen (5 mg/l) was active against *L. innocua* (that was chosen as a surrogate *L. monocytogenes*) in a physiologically neutral solution. Around 99.8% reduction was found over the exposure of 60 seconds in laboratory trials in this study. Short-wave UV (100 $\mu W/cm^2$) decreased the number of *L. monocytogenes* on Tryptose Agar (TA) by seven orders of magnitude in 4 minutes Moreover, age of the culture (48 vs. 24 hours) did not alter the sensitivity of *Listeria* to this UV treatment *(100)*.

C. Chemical Preservatives

Earlier chemical preservatives were used only for food preservation. However, within the last several years interest in using chemicals as antimicrobial agents against foodborne pathogens has increased. Organic acids and fatty acid esters, dimethyl dicarbonate, nitrites, sulfites, etc. are some common chemical antimicrobial agents.

Citric Acid (CA)

There are various parameters that govern the survival and growth of microorganisms in food. The acidity or pH of the food can affect the type and number of microorganism present in a product. Thus, altering the pH or hydrogen ion concentration of food can lead to growth or inhibition of an organism *(26)*. Moreover, incorporation of acids into a food can shorten the thermal process time of foods that are particularly sensitive to changes in sensory qualities, such as texture or appearance. For example, okra, canned in brine containing acetic, citric, lactic, malic, or tartaric acid to achieve an equilibrium pH of 4.3, was processed for 30 minutes in boiling water. Acidification impaired the color, but enhanced the flavor of canned okra. All acids would be effective antibotulinal agents at that pH level *(63)*. There are various organic acids that have been used as an antimicrobial agent in foods. For example, citric acid, acetic acid, lactic acid, malic acid, etc.

Citric acid is a natural antimicrobial agent with a pleasant sour taste. It is affirmed as Generally Recognized As Safe (GRAS) as a direct food substance when used in accordance with good manufacturing practices, in the acid form (21CFR184.1033) and as the calcium (21 CFR 184.1195), potassium (21 CFR 184.1625), or sodium salt (21 CFR 184.1751). it is water highly soluble and enhances the flavor of citrus-based foods. It is approved for use in ice cream,

sherbets and ices, beverages, salad dressings, fruit preserves, and jams and jellies. It is also used as an acidulant in canned vegetables and dairy products *(26)*.

Antimicrobial Properties of CA

The inhibitory capacity of citric, hydrochloric, acetic, lactic, propionic, and phosphoric acid was compared in TSB for *Yersinia enterocolitica* based on concentration of acid, pH, and degree of dissociation. A comparison of equimolar concentrations illustrated citric acid as the most antimicrobial, followed by hydrochloric, lactic, phosphoric, propionic, and acetic acid *(12)*. Inhibition of *L. monocytogenes* was observed in the trypticase soy yeast extract (TSBYE) when the pH of the broth was lowered to 4.0 with citric acid *(21)*. Citric acid at the concentration of 0.5% was effective in complete destruction of viable cells of *A. butzleri* after 8 hour incubation in *Arcobacter* selective broth at 30°C *(72)*. Palumbo and Williams (1994) investigated organic acid dips just prior to packaging as a secondary lethal step to destroy *L. monocytogenes* and observed that combination of 2.5% acetic acid and citric acid restricted the growth and development of *L. monocytogenes* on frankfurters stored vacuum-packaged at 5°C for up to 90 days *(67)*. Through their studies, Sommers and others (2002) demonstrated that citric acid enhanced the susceptibility of *L. monocytogenes* to radiation without compromising frankfurter color, lipid oxidation, firmness, or antioxidant activity *(86)*. Phelps et al. illustrated that addition of 3% citric acid to orange juice caused a 2 log CFU/mL reduction in population of *L. monocytogenes* within 48 hours at 4°C *(71)*. Fischer et al. (1985) used a multiple-hurdle concept and equilibrated hard-cooked eggs with 0.5%, 0.75%, or 1% citric acid and 0.2% sodium benzoate for 30 days at 4°C or in 0.75% citric acid alone for 21 days at 4°C. They observed that 0.75% citric acid was sufficient to reduce inoculated population of *Salmonella* Typhimurium, *Y. enterocolitica*, *E. coli*, and *S. aureus (33)*.

Dimethyl Dicarbonate (DMDC)

Dimethyl dicarbonate (DMDC) is a colorless fruity-smelling liquid with a melting point of 15.2°C and a boiling point of 123-149°C. It is slightly soluble in water and more soluble in organic solvents. DMDC can be used as an 'additive,' to control microbial population, in various beverages such as ready-to-drink tea, wine, and carbonated drinks in accordance with regulations (21CFR172.133). It can be added at a maximum limit of 250 ppm in ready-to-drink tea and various carbonated and non-carbonated beverages. However, in wines, dealcoholized wine, and low alcohol wines its limit is 200 ppm. DMDC inactivates enzymes present in the microbial cells. It is bactericidal at 30-400 μg/mL to a number of species including *Acetobacter pasterurianus*, *E. coli*, *Psedumonas aeroginosa*, *Staphylococcus aureus*, various *Lactobacillus species*, and *Pediococcus cerevisiae* (Hartman, 2003). The inactivation of microorganisms by DMDC is related to the inactivation of the enzymes. Protein modification through reaction of nucleophilic groups, such as imidazoles, amines, or thiols, can readily occur with the dicarbonate *(41)*.

Antimicrobial Properties of DMDC

Studies with DMDC are not very extensive. Fisher and Golden (1998) reported that 0.025% DMDC was most efficient at inactivating *E. coli* O157:H7 ($P<0.05$) at 4 and 10°C when apple cider was treated with 0.025% DMDC, 0.045% sodium benzoate, 0.0046% sodium bisulfite, and combination of the two latter treatments *(34)*. *E. coli* O157:H7 population was reduced from 7 log CFU/mL to undetectable levels at 4°C within 72 hours in apple cider and 48 hours in orange juice containing 250 ppm DMDC *(52)*. Soaking cantaloupes for 3 minutes in a 10,000 ppm DMDC reduced the population of *Salmonella* from 5.01 log CFU/cm^2 to undetectable levels. *Salmonella* population was detected only by enrichment after a 3-minute

treatment with 5000 ppm DMDC *(77)*. DMDC (75 and 150 ppm) was effective in reducing the population of *E. coli* O157:H7 in apple cider by more than 6 log CFU/mL after 24 hour *(76)*. Combination of 500 ppm DMDC with ozone followed by refrigerated storage reduced the number of *E. coli* O157:H7 and *Salmonella* by more than 5 log CFU/mL in apple cider and orange juice *(96)*. However, the same study showed that this treatment was not effective without refrigerated storage.

Hydrogen Peroxide (HP)

Hydrogen peroxide is a very pale blue liquid that appears colorless in a dilute solution and a slightly more viscous than water. It is considered to be GRAS and can be added directly to certain foods for human consumption as an antimicrobial or bleaching agent (21CFR184.1366). HP is a known natural disinfectant and preservative, present in milk, honey, and mucous membranes of the mouth. The most prevalent theory regarding the mechanism of action of HP is the formation of hydroxyl radicals. The hydroxyl radical is highly reactive with microbial membrane lipids, DNA, and other cellular component. Another proposed method is that HP reacts with chloride ions in the cell to produce a hypochlorite ion, a known toxin to microbial cells, and a further reaction of HP and hypochlorite to form a superoxide. The hypochlorite and superoxide have similar reactions with the microbial cells as the hydroxyl radical *(22)*.

Antimicrobial Properties of HP

Hydrogen peroxide is active against wide range of organisms including yeast, bacteria, fungi, viruses, and spores. And thus, one of the major applications of hydrogen peroxide is in sterilizing containers for aseptic processing of foods; e.g., milk, juice, etc. *(11)*. The addition of hydrogen peroxide to milk was encouraged in hot climates where temperature, unsanitary

practices, and poor refrigeration are likely *(25)*. Dominguez and others (1987) determined the effect of the addition of hydrogen peroxide on *L. monocytogenes* present in raw and sterilized milk. They demonstrated that in sterilized milk, 0.0495% hydrogen peroxide was sufficient to product complete destruction of *L. monocytogenes* in 9 hours. However, in raw milk this organism was less susceptible to hydrogen peroxide than milk micro flora. Bell et al. (1997) used 1% acetic acid, 3% hydrogen peroxide, and 1% sodium bicarbonate, alone or in combination, to spray wash beef carcass tissue. They showed that the combination wash of 1% acetic acid with 3% hydrogen peroxide resulted in the greatest reductions of 3.97 and 3.69 $\log_{10}$ CFU/cm^2 for *E. coli* on lean or adipose tissue, respectively. They also showed that this combination of spray wash reduced *L. innocua* by 3.05 $\log_{10}$ CFU/cm^2 on lean tissue and 3.52 $\log_{10}$ CFU/cm^2 on adipose tissue *(7)*. Lin C-M et al. (2002) studied the combined effect of hydrogen peroxide with lactic acid and with mild heat against *E. coli* O157:H7, *Salmonella* Enteritidis, and *L. monocytogenes* on lettuce. They reported that hydrogen peroxide and lactic acid was effective to reduce population of *E. coli* O157:H7 and *Salmonella* Enteritidis by 4 log CFU/g and of *L. monocytogenes* by 3 log CFU/g. However, sensory characteristics of lettuce were compromised with the same treatment. Moreover, they also showed that the treatment of lettuce with 2% hydrogen peroxide at 50°C for 60 seconds was effective in reducing population of these pathogens (≤ 4 log of *E. coli* O157:H7 and *Salmonella* Enteritidis and 3 log of *L. monocytogenes*) and maintaining high product quality. Treatment with 75 and 150 ppm HP in combination with UV reduced *E. coli* O157:H7 population by 7.2 and 7.0 log CFU/mL, respectively, in apple cider after 24 hours of incubation *(76)*. Robbins et al. (2005) illustrated that 3% HP solution reduced the initial concentration of *L. monocytogenes* Scott A planktonic cells by 6.0 log CFU/mL after 10 min of exposure at 20°C, and a 3.5% HP solution reduced the

planktonic population by 5.4 and 8.7 log CFU/mL (complete elimination) after 5 and 10 min of exposure at 20°C, respectively. They also observed that 5% HP reduced the number of cells grown as biofilms by 4.14-log CFU per chip after 10 min of exposure at 20°C and in a 5.58-log CFU per chip (i.e., complete elimination) after 15 min of exposure *(78)*.

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OBJECTIVES

Following are the primary objectives of this research work:

1) Study of the effect of combination of UV and antimicrobials such as citric acid (0.2 and 0.5%), dimethyl dicarbonate (250 and 500 ppm), and hydrogen peroxide (2000 and 4000 ppm) on reducing the number of *L. monocytogenes* in fresh brine.
2) Study of the effect of combination of UV and antimicrobials such as citric acid (0.2 and 0.5%), dimethyl dicarbonate (250 and 500 ppm), and hydrogen peroxide (2000 and 4000 ppm) on reducing the number of *L. monocytogenes* in recycled spent/used brine.

CHAPTER: IV

EFFICACY OF UV AND ANTIMICROBIALS IN REDUCING THE NUMBER OF *LISTERIA MONOCYTOGENES* IN FRESH CHILL BRINE

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Keywords: *Listeria monocytogenes*, brine, UV, citric acid, dimethyl dicarbonate, hydrogen peroxide

ABSTRACT

Chill brines used during ready-to-eat meat processing may be an important source of post-processing contamination by *Listeria monocytogenes* (LM). The purpose of this study was to determine the efficacy of ultraviolet light (UV), antimicrobials such as, citric acid (CA, 0.2 and 0.5%), dimethyl dicarbonate (DMDC, 250 and 500 ppm), and hydrogen peroxide (HP, 2000 and 4000 ppm) alone and in combination with UV to reduce LM in chill brine. Fresh brine (8.0 % w/v NaCl) was inoculated with ~6 log CFU/ml LM cocktail, a specific antimicrobial was added (during antimicrobial treatment), and the solution was exposed to UV-C (~53.8 mJ/cm^2) at -1°C in a circulating tank. Samples were withdrawn at regular intervals for 120 minutes. When LM was no longer detectable via direct plating on MOX, enrichment was performed and colonies were confirmed using API-*Listeria*. Based on LM population reduction rate, UV,UV+500 ppm DMDC, and UV+0.5% CA were found the most effective treatments that required >120, 60, and 45 minutes, respectively, to reach non-detectable level in brine. Thus, based on time UV+0.5% CA was the most effective treatment. Though the reduction rate achieved by 0.5% CA was significantly lower than these treatments, it was as effective as UV+500 ppm DMDC to reach non-detectable level. UV+ 4000 ppm HP required 90 minutes and so was not as effective as UV+500 ppm DMDC and UV+0.5% CA. Higher concentrations of antimicrobials were found more effective than lower concentrations. This work indicates that combinations of UV and antimicrobial may be more effective than either treatment alone except 0.5% CA for the reduction of LM in fresh brines.

INTRODUCTION

Listeria monocytogenes is a halotolerant, Gram-positive, non-spore forming, rod shaped bacterium. It is a foodborne pathogen that causes the illness listeriosis. This organism can grow at temperatures as low as 1.1°C, pH as low as 4.4, and water activities as low as 0.90 *(18)*. Over the last 25 years, listeriosis has been recognized as a major foodborne disease. The economic burden of listeriosis has been estimated to be $255 million per year and associated food recalls were estimated to cost $15 million in 1985 through 1987 *(5)*. Moreover, there are approximately 2000 cases of listeriosis reported every year in the United States, with approximately 500 fatalities *(20)*. *L. monocytogenes* is a significant problem for the food industries due to its ability to survive and grow under adverse conditions (e.g., low temperature, pH, water activity, etc.) that are not tolerated by other non-spore forming foodborne pathogens. However, *Listeria* is susceptible to typical food processes such as cooking, pasteurization, or drying. So, it is primarily an environmental (i.e., food processing environment) contaminant responsible for post-processing contamination of products. Consequently, *L. monocytogenes* is predominantly problematic in ready-to-eat foods; i.e., foods that are not going to be cooked by consumer. Because of the high risk, fatality rate, and cost of listeriosis, it is very important to reduce the risk of this pathogen from foods.

Since *L. monocytogenes* is an environmental contaminant, it is largely associated with food recalls. FSIS reported 8 recalls from January to October 2007 and 34 recalls in a 24 month period starting 2005 *(25)*. These recalls were mainly related to various ready-to-eat meat products, fully cooked chicken products, frozen egg rolls, and sausage products. There are several areas of processing plants where post-processing contamination of products may occur; e.g., slicing, packing, re-bagging, and cooling the product with chilled brine solution. Chill brine

is used to quickly cool thermally processed ready-to-eat products such as frankfurters, deli meats, fish, cheese, etc. to prevent bacterial growth. The USDA has set limits for maximum usage of chill brines based on the salinity and temperature of the brine. Brines may be used for up to four weeks provided that the concentration of salt is 20% with the maximum usage temperature of 10°F *(24)*. *L. monocytogenes* can survive at high salt concentration (~10-12%) and low temperatures (up to 0°C) and, thus, chill brine can harbor this organism once contaminated. Several studies have shown that contamination of ready-to-eat products increases after brining and the most contaminated sites of the processing plant are the brining and post-brining areas *(2, 15)*. Thus, it is very important to prevent *L. monocytogenes* survival in brines.

Previous studies have shown that UV is effective for reducing the population of various microorganisms in water and certain foods *(6, 11, 17, 19, 22, 26, 27)*. Antimicrobial agents such as citric acid, hydrogen peroxide, and dimethyl dicarbonate are effective antimicrobial agents under certain conditions. Therefore, this study is designed to determine the efficacy of UV and antimicrobials alone and in combination for the reduction of *L. monocytogenes* in fresh chill brines.

MATERIALS AND METHODS

Culture Preparation

Three strains of *L. monocytogenes,* Scott A, V7, and L-CDC were used for this study. These strains were obtained from the culture collection at the Department of Food Science and Technology, Virginia Polytechnic Institute and State University. Prior to inoculum preparation, each strain was confirmed using the following method: a portion of each stock culture was transferred to 10 mL of trypic soy broth supplemented with 0.6% yeast extract (TSBYE) and incubated at 35°C for 24 hours. One loopful (10 µL) of 24 h culture was streaked onto Modified Oxford (MOX) agar (Oxford Medium Base and Modified Antibiotic Supplement; Difco, Becton, Dickinson and Company, Franklin Lakes, NJ) for isolation and incubated at 35°C for 48 hours. A presumptive positive colony was selected and streaked again on MOX agar plate that was incubated at 35°C for 24 hours. API *Listeria* strip was inoculated with a colony from the second MOX plate to confirm the identity of the isolate. Once the strain was verified, the confirmed colony of *L. monocytogenes* was transferred from MOX to 25 mL of sterile TSBYE and stock cultures were prepared for the remaining studies.

Stock culture Preparation

Confirmed colony of *L. monocytogenes* was transferred from MOX to 25 mL TSBYE. After 20-22 hours incubation of this broth at 35°C, it was centrifuged and the pallets were obtained. Pallets were re-suspended in fresh sterile TSBYE broth and an equal part of this resuspended culture was combined with sterile TSBYE containing 50% glycerol. This mixture of culture then was mixed well and dispensed 1-1.5mL volume in cryules. These cryules then

stored at -76°C at the Department of Food Science and Technology. This procedure was used to make stock culture of each strain, Scott A, V 7, and L-DCD, of *L. monocytogenes*.

Inoculum Preparation

The stock culture that was prepared was used to prepare inoculum. Before inoculation, the stock cultures were kept in a water bath for few minutes to quickly thaw the culture. A loopful of each purified strain (from stock culture) was transferred separately to TSBYE (25 mL) and incubated at 35°C for 20-22 hours. Twenty mL of each culture was transferred to a centrifuge tube and the mixed culture was then centrifuged, the spent medium was decanted, and the pellet was suspended in 60 mL sterile brine (8% NaCl w/w). Inoculated brine (60 mL) was added to 60 L of test brine to achieve approximately 10^6 CFU/mL initial population.

Brine Preparation

Unused (fresh) chill brine was used in this study. Fresh brine solutions were prepared in the laboratory no more than 24 hours prior to use. All equipment and containers used for brine preparation were cleaned using HC-10 Chlorinated Kleer-Mor high foaming caustic cleaner (EcoLab, St. Paul, MN) and sanitized with Ster-Bac Quaternary Ammonium Sanitizer (200 ppm; EcoLab, St. Paul, MN) to prevent any contamination of brine prior to inoculation. Each container was thoroughly rinsed with tap water to remove any residue of cleaner or sterilizer that may affect the survival of listeriae cells in brine during the treatment. Fresh brines were prepared by dissolving 4.8 kg of Cargill Top-Flow Evaporated Salt (99.8% purity) (Cargill Inc., Minneapolis, MN) into 60 L of tap water to achieve a concentration of 8% w/v NaCl. Each brine solution was placed in a refrigerated room (4°C) before use.

Brine Analysis

Brine was analyzed for its initial pH, salt concentration and background micro-flora. Before adding the inoculum to the brine, a 50 mL brine sample was taken, serially diluted using 0.1% peptone, and plated on Tryptic Soy Agar (TSA). Plates were incubated at 35°C for 48 hours to count the background micro-flora. The salt concentration of brines was measured using hydrometer.

Ultraviolet Light Treatment

An Ultra-Violet Water Treatment Unit (Aquionics Inc., Erlanger, KY), model number AMD 150B/1/2T D was used for this research (Figure 4.1). The system is effective in the most challenging applications including treating super cooled water, hot water, poor transmission fluids, such as brine, and high dose situations *(1)*.

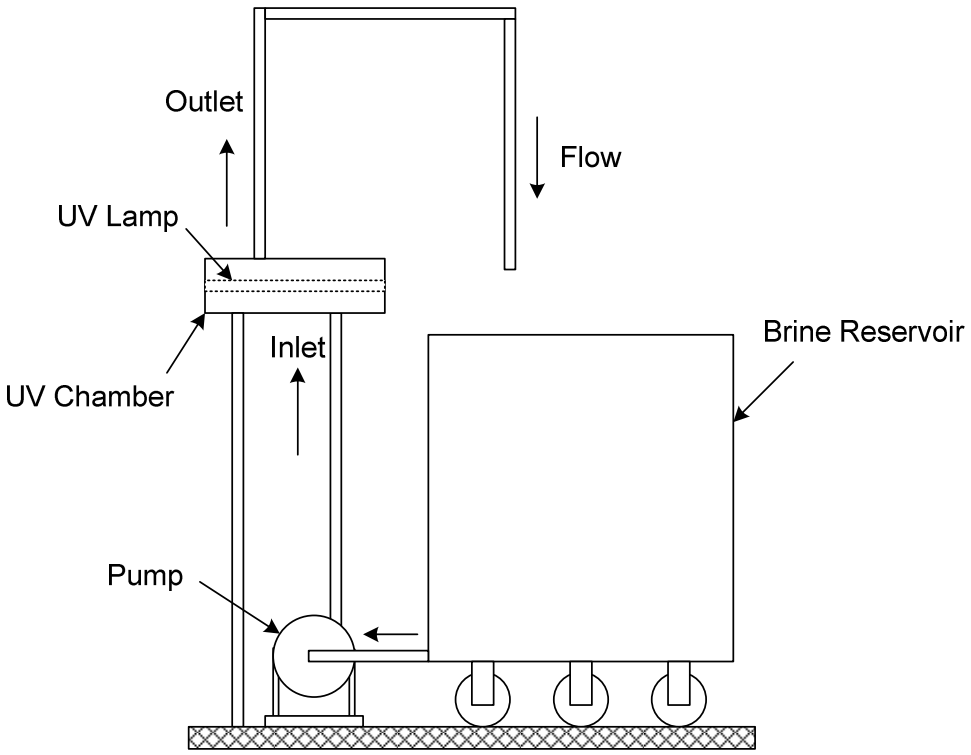

Figure 4.1: Ultra-Violet Water Treatment Unit

The brine was treated by passing it through a stainless steel chamber containing single, broad spectrum, medium pressure UV lamp. The lamp was mounted in a quartz sleeve and fitted within the chamber allowing brine to pass the sleeve on all sides. A diameter of the lamp quartz was 20.5 mm and the arc length was 280 mm. The UV system was additionally connected with an in-line chiller unit (GCI Icewagon) to keep the brine temperature of 30°F (-1°C) during processing. Moreover, entire UV treatment system was kept in a 4°C laboratory in order to allow the brine to remain chilled. After the UV exposure, some of the brine would return to a reservoir directly and some would return to reservoir via chiller. Thus, it was continually pumped through the UV system during the entire treatment time.

Brine prepared as described above was then exposed to UV at a peak output of 254 nm with an approximate UV dose of 53.8mJ/cm^2. Since the brine was prepared using the Blacksburg municipal water supply, it contained chloramines. In order to decompose chloramines, the brine was first placed in the reservoir of the system and recycled with UV exposure. The UV lamp was then turned off and a 50 mL sample was taken for analysis of background microflora. Then the brine was inoculated with the cocktail of three *L. monocytogenes* strains and circulated to mix. The approximate inoculum was 6.0 log CFU/mL. The inoculated brine then was exposed to UV for up to two hours with the flow rate of 198 L/min. Samples (50 mL) were taken at 0, 1, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes and analyzed for *L. monocytogenes*. All the samples were taken in sterile 90 mL specimen containers (Fisher Scientific, Inc) to avoid sample contamination. To see the effect of the UV system flow and temperature, inoculated brine was circulated through the system with the lamps turned off that served as control for all the treatments.

After each run, the UV system was cleaned using HC-10 Chlorinated Kleer-Mor high foaming caustic cleaner (EcoLab, St. Paul, MN) and sanitized with Ster-Bac Quaternary Ammonium Sanitizer (200 ppm; EcoLab, St. Paul, MN) to destroy any remaining listeriae cells and to prevent any contamination of next batch of brine.

Antimicrobial Treatment

Inoculated brine was treated with antimicrobial agents, such as citric acid (0.1% and 0.2%), hydrogen peroxide (2000 ppm and 4000 ppm), and dimethyl dicarbonate (DMDC) (250 ppm and 500 ppm) in the absence of UV. The concentration of each antimicrobial was selected through a preliminary study. Brine was inoculated with approximately 6.0 log CFU/mL *L. monocytogenes* cocktail and circulated for one minute. Respective antimicrobial agent was then added and zero-minute sample was taken. Brine was circulated through the system with added antimicrobial for 120 minutes. Samples were withdrawn at the same interval as UV treatment and were analyzed for detection of *L. monocytogenes*

Combination of UV and antimicrobial Treatment

Brine inoculated with the test organism was also treated with combination of UV and antimicrobial agents. For this treatment, a specific antimicrobial in a specific concentration was added in inoculated brine and a sample was withdrawn that was called zero-minute sample. Immediately after that the UV was turned on and brine was processed with UV and added antimicrobial for 120 minutes. Samples were taken at the same interval as UV treatment and were subjected to microbiological analysis for *L. monocytogenes*.

Microbiological analysis

Samples of test brines were serially diluted using 0.1% peptone and plated on to duplicate MOX agar plates followed by 48 hours incubation at 35°C. Typical black colonies were considered as presumptive for *L. monocytogenes*. After incubation, colonies were counted and results were reported in CFU/mL. An enrichment procedure was also performed to detect the cells once population reach to detection limit (1.00 log CFU/mL) and are no longer recoverable by direct plating. For this purpose, 50 mL Brain Heart Infusion (BHI; Difco, Becton Dickinson and Company, Franklin Lakes, NJ) broth was inoculated with the 10 mL brine sample followed by 48 hours incubation at 35°C. After 24 hours, a loop-full of culture from BHI was streaked on MOX plates followed by 48 hours incubation at 35°C. Then, results were reported in presence or absence of *L. monocytogenes* in the samples. Typical colonies on MOX were confirmed using *Listeria* API.

Statistical Analysis

Each experiment of UV and combination of UV and each antimicrobial were replicated three times for accurate results. The data of direct plating were analyzed using JMP statistical software version 6.00 (SAS Institute; Cary NC). For these quantitative data, regression analysis was performed to calculate slope and so the rate of *L. monocytogenes* reduction in brines. Since the regression analysis was performed on each replication of all the treatments, there are more than one statistical models of this study. An example of these models for one of the treatments used in fresh and spent brine is described in detail in Appendix A. The mean rate of reduction was then obtained using one way ANOVA, where the value of α equaled 0.05. The results of enrichment studies were qualitative that described either presence or absence of *L.*

monocytogenes in samples. Therefore, they were used to illustrate Time (in minutes) to reach a non-detectable level in brines, and therefore, the total processing time.

RESULTS

The results of brine analysis showed that the average pH value of fresh brine was 7.1 with average salt concentration of 8.0%. The results of total aerobic plate count illustrated that population of background micro-flora (aerobic mesophiles) in fresh brine was generally non-detectable; i.e., below 1.0 log CFU/mL.

The microbiological analysis of brine was performed to observe the trend of *L. monocytogenes* inactivation through various treatments in brine. Through the experiments, two types of data sets were generated, (i) quantitative data from the results of direct surface plating and (ii) qualitative data through enrichment studies. Results of direct surface plating on MOX are shown in figures 4.2, 4.3, 4.4, and 4.5. These results mainly explain the inactivation trend of *L. monocytogenes* in response to all treatments.

Table 4.1 illustrates the fate of *L. monocytogenes* during the UV treatment of fresh brine containing citric acid, dimethyl dicarbonate, and hydrogen peroxide. The average starting population of *L. monocytogenes* for all treatments was 5.7 log CFU/mL of brine. The slope of all treatments was negative, which suggests that the number of *L. monocytogenes* decreased with time.

Based on the rate of reduction of *L. monocytogenes*, we can sort the treatments from the highest to lowest rates. Note that there are treatments that have a rate of reduction that are not significantly different than other treatments. Such treatments were grouped together and given the same rank (i.e., same letter as a superscript in Table 4.1). The final ranking is as follows: UV+500 ppm DMDC, UV, UV+0.5% and CA UV+0.2% CA; UV+250 ppm DMDC, UV+2000 ppm HP, 0.5% CA and UV+4000 ppm HP, 0.2% CA, 500 ppm DMDC, 250 ppm DMDC, 2000 ppm HP, 4000 ppm HP, and Control.

In contrast, based on the time (in minutes) to reach a non-detectable levels of *L. monocytogenes* through the enrichment study, we can sort the treatments from the lowest to highest required time as UV+0.5% CA; 0.5% CA and UV+500 ppm DMDC; UV+250 ppm DMDC and UV+4000 ppm HP; UV+2000 ppm HP; 4000 ppm HP; followed by the remaining treatments. Since the study was performed for 120 minutes, all treatments that required more than 120 minutes were given the same rank.

Results of the control study showed that both low temperature and brine circulation through the system did not have any negative effect on the survival of *L. monocytogenes*, and that the population was constant throughout the 120-minute time-period. When fresh brine was treated with UV alone, *L. monocytogenes* population decreased below the detection limit (i.e., <1 log CFU/mL) through surface plating in 15 minutes (Figure 4.2) with a reduction rate of 0.87 log CFU/mL per minute (Table 4.1). However, *L. monocytogenes* was detectable by enrichment throughout the 120-minute time-period. This shows that enrichment plays a significant role for the selection of any method to maintain a zero tolerance policy of *L. monocytogenes* in ready-to-eat products.

When UV treatment was combined with 500 ppm DMDC the population of *L. monocytogenes* reduced from 6.10 log CFU/mL to below the detection limit through surface plating in 15 minutes and achieved the highest rate of reduction (0.90 log CFU/mL per minute). The reduction rate achieved by UV treatment was not significantly different from UV+500 ppm DMDC. However, the latter treatment required only 60 minutes to reduce the listeriae population to a non-detectable level through enrichment. On the other hand, when brine was treated only with 500 ppm DMDC it required 90 minutes to reduce the population below detection limit through direct surface plating (Figure 4.2) and more than 120 minutes to reduce

the population to a non-detectable level through enrichment (Table 4.1). Similarly, the combination of UV and 250 ppm DMDC was more effective than either of the treatments alone. UV+250 ppm DMDC and 250 ppm DMDC alone required 15 and 105 minutes, respectively, to reduce the number of listeriae cells from 6.0 log CFU/mL to less than the detection limit (Figure 4.2). Moreover, cells were recovered until 75 and 120 minutes through enrichment with UV+250 ppm DMDC and 250 ppm DMDC, respectively (Table 4.1). This confirms that antimicrobial agents, such as DMDC, improve the efficacy of UV and that the multiple hurdle technology works better than using one hurdle/barrier in pathogen reduction *(14, 21)*.

The combination of UV with 0.5% CA reduced the listeriae population from 5.98 log CFU/mL to less than the detection limit in 15 minutes (Figure 4.3) with a mean rate of decline of 0.79 log CFU/mL per minute (Table 4.1). The reduction rate of UV+0.5% CA treatment was not significantly different from UV and UV+500 ppm DMDC treatments ($P>0.05$). However, UV+0.5% CA required only 45 minutes in order to reach a non-detectable level through enrichment, which was lower than the time required by UV+500 ppm DMDC. Therefore, UV+0.5% CA may be better than UV+500 ppm DMDC to reduce the population of *L. monocytogenes* in relatively short time.

The enrichment studies also showed that 0.5% CA treatment required as much time as UV+500 ppm DMDC (60 minutes) to reach the non-detectable level. However, the reduction rate of 0.5% CA Control (0.31 log CFU/mL per minute) was significantly lower than UV+500 ppm DMDC (0.90 log CFU/mL per minute). Thus, according to total time required to process brine to achieve non-detectable level in brine, UV+0.5% CA was the most effective treatment. In contrast, 0.2% CA did not improve the efficacy of UV and was not found to be one of the effective treatments to process brine (Table 4.1).

It was also observed that UV+4000 ppm HP was not so effective when compared to UV+500 ppm DMDC and UV+0.5% CA. Addition of HP reduced the rate achieved by UV alone and so UV+HP took more time than UV to reduce the population below detection limit through direct plating. UV+2000 ppm HP and UV+4000 ppm HP required 15 and 30 minutes, respectively, to achieve a non-detectable level. However, they required shorter time, 90 and 105 minutes, respectively, to reach a non-detectable level through enrichment than UV alone (Figure 4). Moreover, enrichment studies showed that 4000 ppm HP alone required 120 minutes to reach a non-detectable level in brine. Therefore, among all the antimicrobial treatments without UV, 0.5% CA was the most effective treatment followed by 4000 ppm HP. With all other antimicrobial treatments listeriae cells were recovered up to 120 minutes through enrichment.

DISCUSSION

L. monocytogenes has recently emerged as one of the major etiological agents of foodborne disease. It has caused a significant problem with food recalls that has affected the economy of the country *(7-10, 25)*. Because of the high fatality rate and the low infective dose of *L. monocytogenes* there is a zero tolerance policy of pathogen in ready-to-eat food products. It has also been observed by USDA that *L. monocytogenes* is primarily associated with various recalls of fully cooked, ready-to-eat, and frozen products. Since chill brine is used to quickly cool thermally processed ready-to-eat products, it is a potential point in the process where *L. monocytogenes* contamination may occur. However, not much work has been done on processing of brine to reduce or remove this pathogen. Therefore, the main objective of this study was to use a combination of non-thermal processes, such as UV and antimicrobials, to reduce the population of *L. monocytogenes* in fresh chill brines.

This research showed that when fresh brine was treated with UV the *L. monocytogenes* population decreased significantly, by 5.0 log CFU/mL, in 15 minutes of UV-exposure. This finding is in accordance with Gailunas (2003) and Quicho (2005). Gailunas (2005) reported reduction of around 4.0 log CFU/mL listeriae cells within 15 minutes in 7.9% and 13.2% brines *(13)*. Quicho (2005) observed 4.7 log CFU/mL reduction of *E. coli* in apple cider immediately after UV exposure *(21)*. However, cells were recovered up to 120 through enrichment. This explains the sigmoidal curve produced by UV. Researchers have observed that UV reduces microbial population exponentially and so produces a sigmoidal curve *(3, 23, 28)*. This tailing effect may be due to a lack of homogenous distribution of population in brine. The tailing effect is also explained by multiple hit phenomenon described by Yousef and Marth *(28)*. According to

this phenomenon multiple UV hit on a single cell or single UV hit on multiple cells produces a sigmoidal survival curve. Addition of antimicrobials, however, reduced the tailing effect that was observed by UV.

The addition of 500 ppm DMDC and 0.5% CA did not significantly affect the reduction rate achieved by UV alone and required similar time to reduce the population below detection limit through direct plating. However, enrichment studies showed that the combined treatment of UV and 0.5% CA was the most effective in reducing the number of *L. monocytogenes* to a non-detectable level in short time. This means that DMDC needs a bit more contact time than CA to achieve similar results. When comparing the lower concentration levels of DMDC and CA the reduction rate of UV+250 ppm DMDC was significantly lower than UV+0.2% CA. However, the listeriae cells were recovered up to 120 minutes through enrichment with the latter treatment, whereas former treatment required 90 minutes. Thus, DMDC was more effective than CA at lower concentrations.

The addition of HP negatively affected the reduction rate achieved by UV and so UV+HP required more time than UV to reduce the population below the detection limit through direct plating. This may be due to HP's UV absorption property. It is known that HP absorbs UV when it ranges from 200-300 nm *(16)*. This may have caused low UV availability to destroy microbial cells. On the whole, the combination of UV and 4000 ppm HP was found relatively less effective in fresh brine among other combinations of UV (i.e., UV with 500 ppm DMDC and 0.5% CA). This may be explained by the instability of HP in brine solution and effect of pH on antimicrobial property of HP. It has been studied that HP shows better antimicrobial activity in acidic pH and slightly lower activity in neutral pH. However, HP's overall effectiveness is diminished at alkaline pH *(12)*. Baldry *(4)* studied the effect of pH on antimicrobial properties

of HP and showed that 3% solution of HP killed *Bacillus subtilis* spores in 3 hours at pH 5. However, the same solution required 6 hours at pH 6.5 and pH 8.0. Thus, a neutral pH (7.1) of the brine may have reduced the antimicrobial property of HP in this study and so it was not found as effective as UV+500 ppm DMDC and UV+0.5% CA.

When the combination of UV with lower concentration of HP (2000 ppm) was compared to that of DMDC (250 ppm) and CA (0.2%) the reduction rate of UV+2000 ppm HP was significantly lower than UV+0.2% CA ($P>0.05$) and was not significantly different from UV+250 ppm DMDC. However, UV+2000 ppm HP required 105 minutes to reduce the population below non-detectable levels, which was slightly higher than UV+250 ppm DMDC (90 minutes) and lower than UV+0.2% CA (>120 minutes). Thus, though the reduction rate of UV+0.2% CA was higher than the other two treatments, the latter treatments were found more effective based on time. Among the lower concentrations of antimicrobials UV+250 ppm DMDC was found slightly better than UV+2000 ppm HP; the latter was found slightly better than UV+0.2% CA.

Overall, antimicrobial treatments alone were not as effective as the combination of UV and antimicrobials except 0.5% CA that required 60 minutes to reach a non-detectable level in brine. 0.5% CA treatment was found almost equally effective with and without UV. Another antimicrobial that was found effective was 4000 ppm HP that required 120 minutes to reach a non-detectable level. Thus, 0.5% CA and 4000 ppm HP may be effective to use alone in reducing the *L. monocytogenes* population in fresh brine. In summary, this research showed that a combination of UV and antimicrobials was more effective in reducing the population of *L. monocytogenes* to a non-detectable level in brine than any of the treatment alone except the

treatment of 0.5% CA. Moreover, higher concentration of antimicrobials were, generally, more effective than lower concentrations.

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Table 4.1: Fate of *Listeria monocytogenes* during Ultraviolet Light Treatment of **Fresh Brines** Containing Citric Acid (0.2 and 0.5%), Dimethyl Dicarbonate (250 and 500ppm), and Hydrogen Peroxide (2000 and 4000ppm) in a Recirculating Brine Chiller System.

Treatment	Mean Rate of Reduction (Log CFU/mL per minute)	Average R^2	Time (min.) to Reach ND*
Control	4.5×10^{-4} [a]	0.21	>120
4000ppm HP	0.04 [a]	0.94	120
2000ppm HP	0.04 [a]	0.98	>120
250ppm DMDC	0.06 [a]	0.75	>120
500ppm DMDC	0.06 [a]	0.90	>120
0.2% CA	0.12 [a]	0.93	>120
UV + 4000ppm HP	0.16 [a]	0.74	90
0.5% CA	0.31 [a,b]	0.97	60
UV + 2000ppm HP	0.32 [a,b]	0.79	105
UV + 250ppm DMDC	0.32 [a,b]	0.75	90
UV + 0.2% CA	0.57 [b,c]	0.97	>120
UV + 0.5% CA	0.79 [c]	0.97	45
UV	0.87[c]	0.99	>120
UV + 500ppm DMDC	0.90 [c]	0.99	60

Means followed by the same letter are not significantly different at the 0.05 level (experiment wise), using Tukey's HSD Test.

*ND = Non Detectable level; the organism did not recover through enrichment.

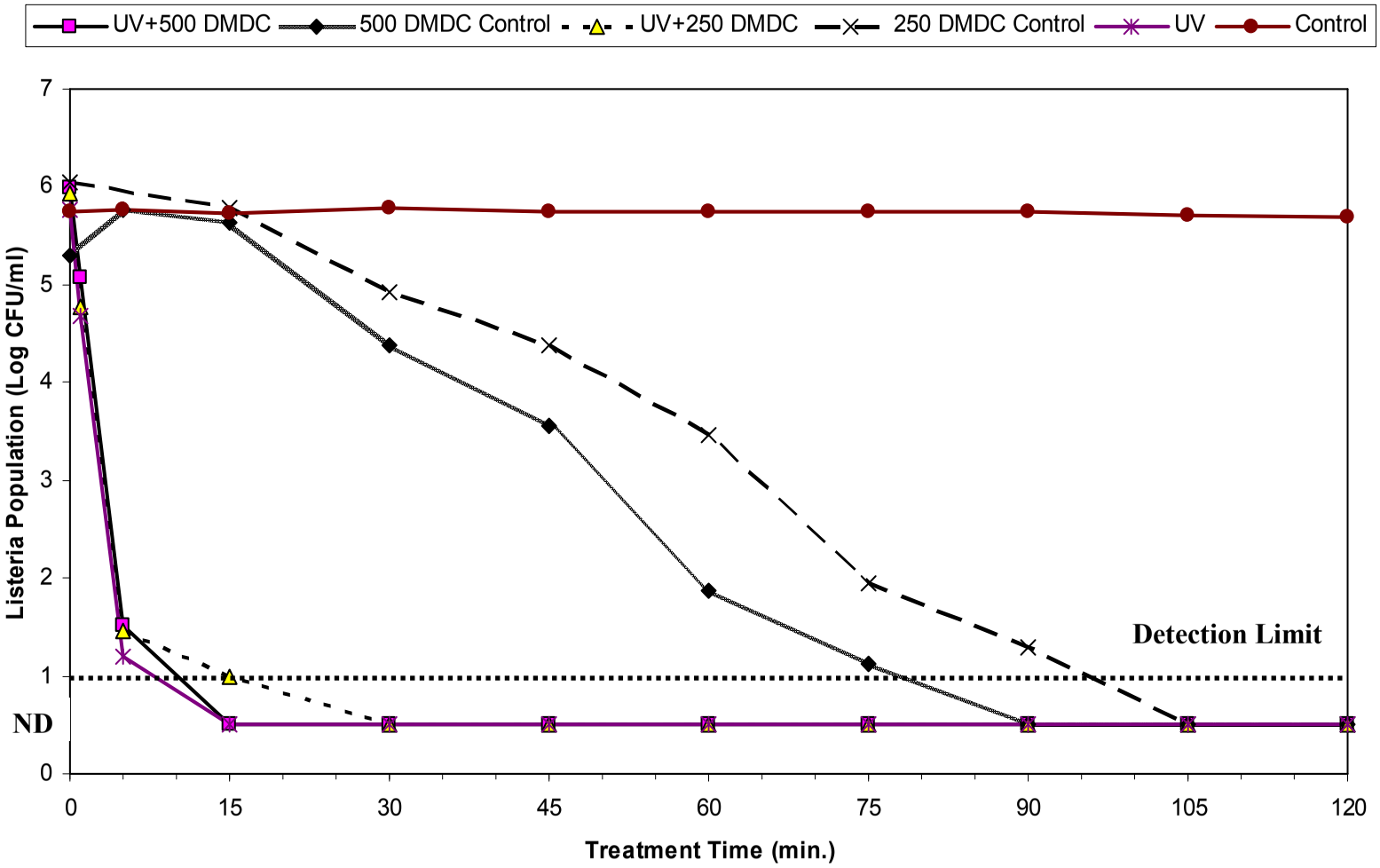

Figure 4.2: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with DMDC (250 and 500 ppm) treatments in Fresh Brine. ND = Not Detected; the organism did not recover through direct surface plating.

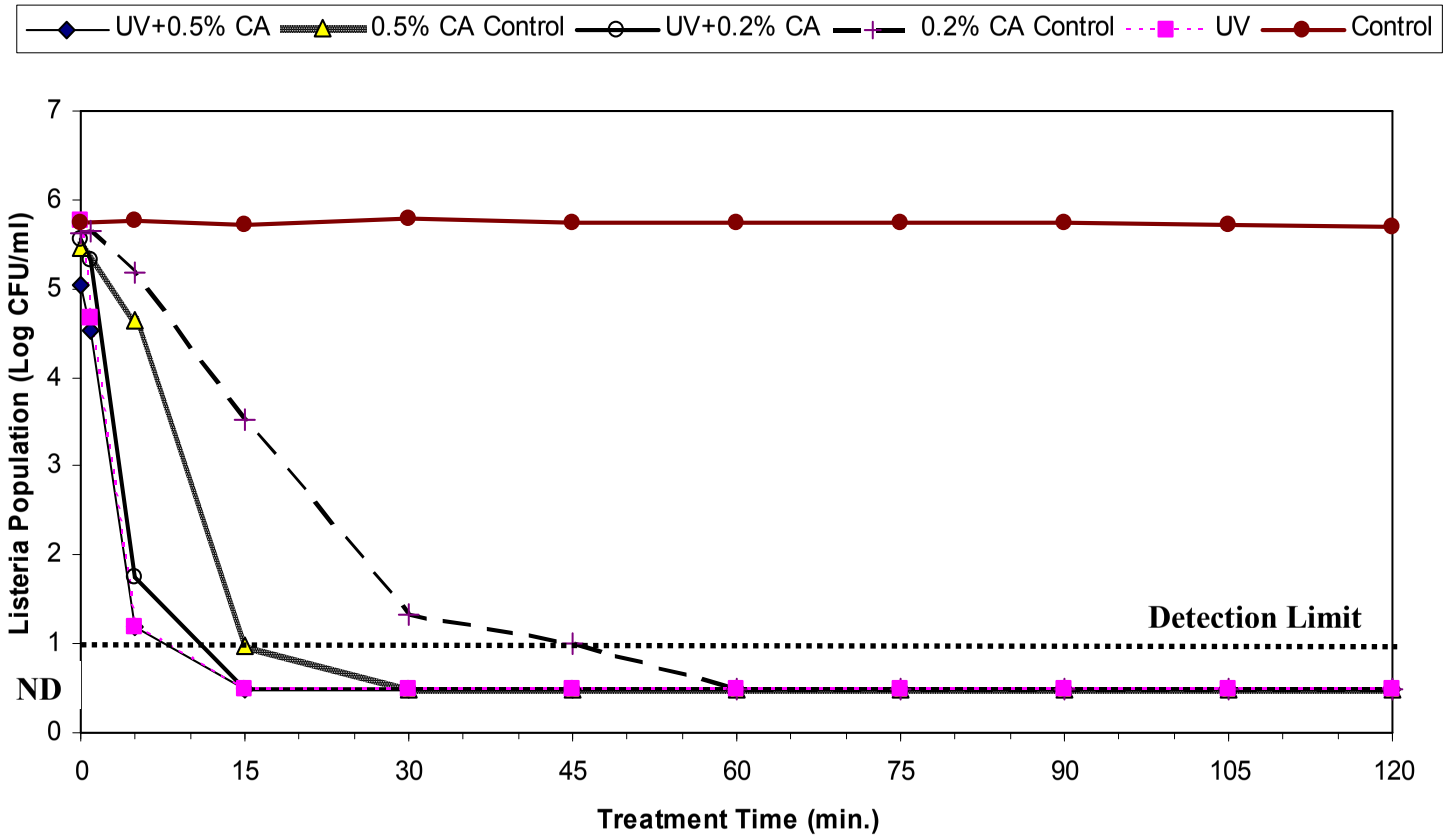

Figure 4.3: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with CA (0.2 and 0.5%) treatments in Fresh Brine. ND = Not Detected; the organism did not recover through direct surface plating.

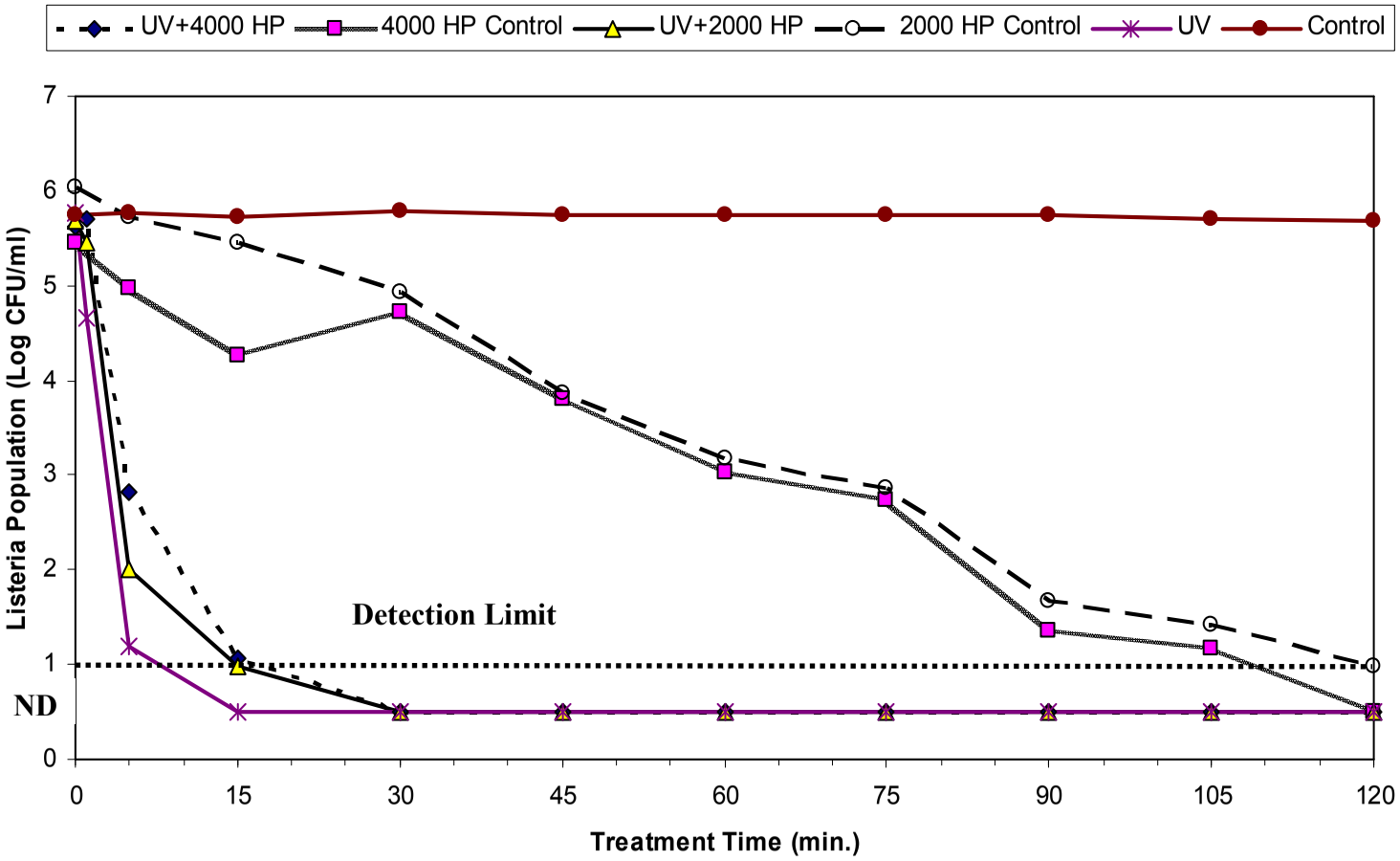

Figure 4.4: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with HP (2000 and 4000 ppm) treatments in Fresh Brine. ND = Not Detected; the organism did not recover through direct surface plating.

CHAPTER: V

REDUCING THE NUMBER OF *LISTERIA MONOCYTOGENES* IN RECYCLED SPENT CHILL BRINE USING ULTRAVIOLET LIGHT AND ANTIMICROBIALS

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Keywords: *Listeria monocytogenes*, recycled brine, UV, citric acid, dimethyl dicarbonate, hydrogen peroxide

ABSTRACT

The U.S. Food Safety and Inspection Services (FSIS) reported several *Listeria* recalls in the last 2 years. These recalls were mainly for various fully cooked, ready-to-eat, or frozen meat and sausage products. Post-processing contamination of the products in a processing plant has been identified as one of the major reasons for food contamination with *Listeria*; brining is one such post-processing area. This study focused on the reduction of *L. monocytogenes* in spent/used brine through the "multiple hurdle technology." Spent (recycled) brine obtained from a frankfurter processor was used in the study. Specifically, UV, antimicrobial agents, such as citric acid (CA), hydrogen peroxide (HP), and dimethyl dicarbonate (DMDC), and their combinations were used. Results of this study show that the combinations of UV and 2000 ppm and 4000 ppm HP were the most effective treatments in reducing the *Listeria* population in a relatively short time (120 minutes). Both these treatments were found to be more effective than the treatments that used UV alone and HP alone. Additionally, all other treatments, such as the combinations of UV and CA (0.2% and 0.5%), and UV and DMDC (250 ppm and 500 ppm) were comparatively less effective. This may be due to the presence of organic matter in spent brine, which may have reduced the penetration of UV and availability of antimicrobials for microbial interaction.

INTRODUCTION

Listeria monocytogenes has been primarily associated with food recalls. The U.S. Food Safety and Inspection Services (FSIS) recently reported 8 *Listeria* recalls in a 10-month period starting January 2007; a total of 34 recalls were reported in a 24-month time period starting 2005. These recalls were mainly for various fully cooked, ready-to-eat, or frozen meat products, and sausage products *(25)*. An estimated cost of food recalls was $15 million in 1985 through 1987 *(4)*. Thomsen el al. *(22)* measured the sales losses experienced by frankfurter brands following a recall for *L. monocytogenes*. Their results indicate that sales of recalled brands declined roughly 22% after a recall. Furthermore, this organism has about 25% fatality rate *(18)* and has also been responsible for several foodborne outbreaks in the past *(6-10)*.

One of the major reasons for food contamination with *Listeria* is post-processing contamination of the products in a processing plant. For example, in frankfurter processing cellulose casing is mechanically peeled off with a knife after cooking and then the cooked frankfurters are transferred through conveyors. So if the knife, conveyor, or any of the packaging materials are contaminated with *Listeria*, then the cooked ready-to-eat frankfurters may be contaminated with this organism *(20, 23)*. Moreover, this pathogen can survive in adverse conditions, such as low pH, temperature, water activity, and high salt concentration. It can, therefore, survive in contaminated ready-to-eat foods. Such a contamination may pose a high risk as consumers do not cook these products before consumption. Furthermore, the risk of foodborne listeriosis increases if the contaminated products are exposed to temperature abuse.

According to the U.S. Department of Agriculture (USDA), contamination of foods with *L. monocytogenes* is a hazard that is likely to occur in all ready-to-eat meat and poultry products that are exposed to post-lethality processing environment. The FSIS, therefore, issued an interim final rule to protect consumers from foodborne listeriosis. According to this rule all ready-to-eat meat manufacturers in the U.S. are required to adopt one of the three alternatives to effectively control the risks posed by *L. monocytogenes (16)*. These alternatives are the following: (i) the use of a post-lethality treatment *and* an antimicrobial agent (or process) to suppress or limit the growth of this microorganism; (ii) the use of a post-lethality treatment *or* an antimicrobial agent (or process) to suppress or limit the growth of this microorganism; and (iii) controlling *L. monocytogenes* in the post-lethality environments strictly through sanitary procedures. These post-lethality processing environments, according to USDA, are the areas into which products are routed after complete thermal treatments. These areas include slicing, peeling, dicing, re-bagging, and brining *(13)*.

Larson et al. *(17)* reported survival of *L. monocytogenes* in commercial cheese brines where the concentration of salt (NaCl) ranged from 5.6 to 24.7%. That is, brine can harbor this organism once contaminated. Several studies have also shown that contamination of ready-to-eat products increases after brining and the most contaminated sites of the processing plant are the brining and post-brining areas *(2, 14)*. Thus, it is very important to prevent *L. monocytogenes* survival in brines especially when they are recycled.

Chill brine is a concentrated salt solution that is used to quickly cool cooked ready-to-eat products such as frankfurters, deli meats, fish, cheese, etc., to prevent bacterial growth. Chill brine is often recycled, which helps reduce the cost of food processing. However, the USDA has set some regulations on recycling of brine based on the salinity and temperature of brine. Under

certain conditions brine may be used for up to four weeks. Table 5.1 summarizes the required temperature and salt concentration for recycling of brine used for heat treated products *(24)*.

Table 1: USDA regulations for recycled chill brine

Maintenance Conditions for Brine Used for Heat Treated Products		
Duration of Use	*Minimum salt concentration (%)*	*Maximum temperature*
One production shift	None	None
Up to 24 hours	5	40°F
Up to 1 week	9	28°F
Up to 4 weeks	20	10°F

Parikh et al. *(19)* observed that a combination of UV and antimicrobial agents such as citric acid, hydrogen peroxide, and dimethyl dicarbonate was effective at reducing *L. monocytogenes* population in fresh brine. However, it is also important to observe the effect of such combinations in used/spent brine since brine solutions are generally recycled in industries. Therefore, this study is designed to determine the efficacy of UV and antimicrobials for the reduction of *L. monocytogenes* in recycled spent chill brines.

MATERIALS AND METHODS

Culture Preparation

The *L. monocytogenes* strains that were used in this study were obtained from the culture collection at the Department of Food Science and Technology, Virginia Polytechnic Institute and State University. Cocktail of Scott A, V7, and L-CDC were used and each strain was confirmed prior to use. Following method was used for strain confirmation: three tubes of 10 mL of TSBYE (trypic soy broth supplemented with 0.6% yeast extract) was inoculated with a loopful (10 μL) of each strain stock culture and incubated at 35°C for 24 hours. The culture then was streaked onto Modified Oxford (MOX) agar (Oxford Medium Base and Modified Antibiotic Supplement; Difco, Becton, Dickinson and Company, Franklin Lakes, NJ) for isolation and incubated at 35°C for 48 hours. To obtain a pure culture, single presumptive positive colony was selected and streaked again on MOX agar plate and was incubated at 35°C for 24 hours. The isolate then was confirmed using API *Listeria* strip. After verification of each strain, the confirmed colony of *L. monocytogenes* was transferred from MOX to 25 mL of sterile TSBYE and stock cultures were prepared, which was used for the remaining studies.

Stock culture Preparation

TSBYE (25 mL) was inoculated with confirmed colony of *L. monocytogenes* and incubated for 20-22 hours 35°C. The broth was then centrifuged to obtain pallets. Pallets were re-suspended in fresh sterile TSBYE broth and an equal part of this resuspended culture was combined with sterile TSBYE containing 50% glycerol and mixed well. This mixture of culture was then dispensed in 1-1.5 mL volume in cryules. These cryules were stored at -76°C at the

Department of Food Science and Technology. This procedure was used to make stock culture of each strain, Scott A, V 7, and L-DCD, of *L. monocytogenes*.

Inoculum Preparation

For inoculum preparation, a loopful of each purified strain (from stock culture) was transferred to different TSBYE (25 mL) and incubated at 35°C for 20-22 hours. Twenty mL of each culture was transferred to a centrifuge tube to make final volume of 60 mL. The mixed culture was then centrifuged, the spent medium was decanted, and the pellet was suspended in 60 mL sterile brine (8% NaCl w/v). Sixty liters of test brine was inoculated with this 60 mL inoculum to achieve approximately 6 log CFU/mL initial population.

Brine Analysis

Recycled spent brine was obtained from a frankfurter processor after its maximum usage. Each batch of spent brine (approximately 1000 L) was shipped via refrigerated truck and stored at 4°C until use. It was analyzed for its initial pH, salt concentration and background micro-flora. Before adding the inoculum to the brine, a 50 mL brine sample was taken to measure background micro-flora and salt concentration. For analysis of background micro-flora the sample was serially diluted using 0.1% peptone, and plated on Tryptic Soy Agar (TSA). Plates were incubated at 35°C for 48 hours to count the colonies. The salt concentration of brines was measured using Quantab Chloride Titrators (Hach Chemical Co., Matthews, NC). Moreover, spent brine was analyzed for total solids and total suspended solids using Standard Methods for the Examination of Water and Waste Water as mentioned below:

Analysis of Total Solids (TS)

For Total Solids (TS) the standard method of water and waste water treatment (2540 B) was used *(11)*. A specific amount of well-mixed sample was dispensed in a pre-weighed dish. The sample dish was kept in an oven at around 98°C for some time (approx. 35-40 min) to prevent splattering while evaporating water from the sample. Then the sample was dried at 103-105°C until constant weight was achieved. Samples were analyzed in triplicate from each batch of new spent brine. The total solids can be calculated using following equation:

$$\text{Mg total solids/L} = (A-B) \times 1000/ \text{ sample volume (mL)},$$

where A = weight of dried residue + dish, mg, and B = weight of dish, mg.

Analysis of Total Suspended Solids (TSS)

Total Suspended Solids (TSS) was measured using the standard method for water and waste water (2540 D) *(11)* and was analyzed in triplicate from each batch of spent brine. A well-mixed sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter was dried to a constant weight at 103-105°C. The increase in weight of the filter represents the total suspended solids that can be calculated using following equation:

$$\text{Mg total suspended solids/L} = (A-B) \times 1000/ \text{ sample volume (mL)},$$

where A = weight of filter + dried residue, mg, and B = weight of filter, mg.

Ultraviolet Water Treatment Unit

An Ultra-Violet Water Treatment Unit (Aquionics Inc., Erlanger, KY), which was used for the fresh brine study *(19)* was also used for this research (Figure 5.1). The model number of the UV treatment unit was AMD 150B/1/2T D. This UV treatment unit is effective with super cooled water, hot water, poor transmission fluids, such as brine, and high dose situations *(1)*.

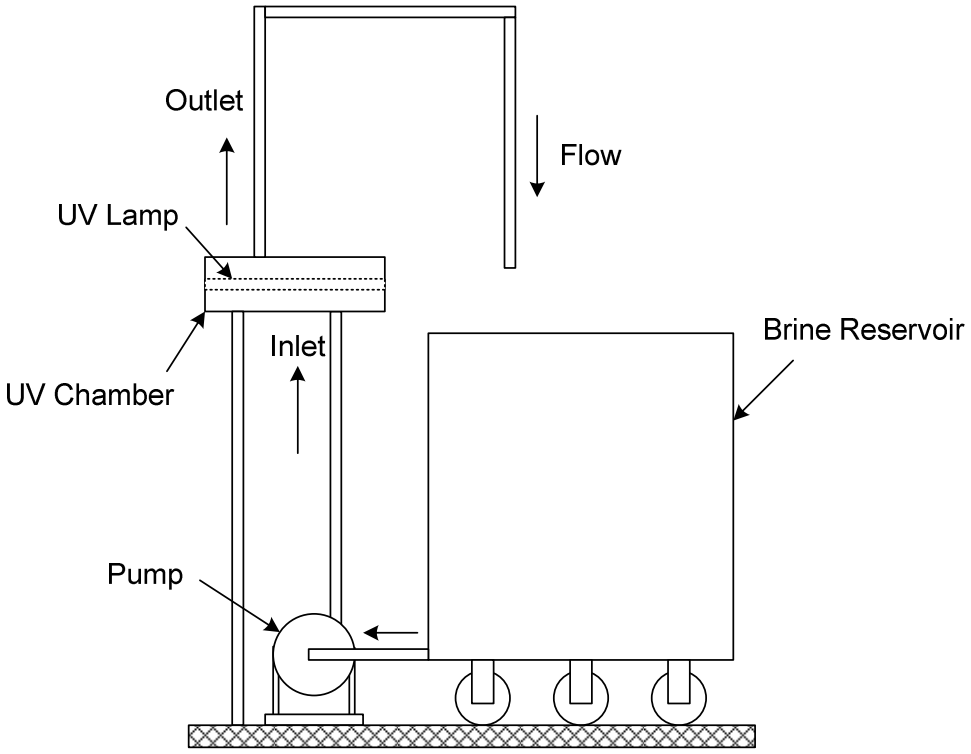

Figure 5.1: Ultra-Violet Water Treatment Unit

The brine was exposed to a peak output of 254nm UV that produced an approximate UV dose of 53.8mJ/cm^2. UV lamp was mounted in a quartz sleeve that was placed in a stainless steel

chamber so that brine could circulate through all the sides of the lamp. A diameter of the lamp quartz was 20.5 mm and the arc length was 280 mm. The brine temperature was maintained at 30°F (-1°C) during processing by connecting the UV system (kept in a 4°C laboratory) with an in-line chiller unit (GCI Icewagon). After the UV exposure, a portion of brine was allowed to return to a reservoir directly, while the remaining portion was allowed to return to the reservoir via a chiller. That is, the brine was continually pumped through the UV system during the entire treatment time.

UV Treatment of Brine

For this entire research, 60 L of brine was used that was kept in brine reservoir and allowed to circulate until the temperature of brine was reduced to 30°F. Then a 50 ml sample was taken for analysis of background microflora, pH, and salt concentration. The brine was inoculated with a cocktail of three *L. monocytogenes* strains (approximately 6.0 log CFU/mL) and circulated to mix. The inoculated brine then was exposed to UV for up to 120 minutes with the flow rate of 198 L/min. Samples (50 mL) were taken at 0, 1, 5, 15, 30, 45, 60, 75, 90, 105, and 120 minutes and analyzed for *L. monocytogenes*. All the samples were taken in sterile 90 mL specimen containers (Fisher Scientific, Inc) to avoid sample contamination. To determine the effect of UV unit, flow rate, and low temperature on survival of *L. monocytogenes* the inoculated brine was circulated through the UV system with the lamps turned off that served as control for all the treatments. After each run, the UV unit was cleaned using HC-10 Chlorinated Kleer-Mor high foaming caustic cleaner (EcoLab, St. Paul, MN) and sanitized with Ster-Bac Quaternary Ammonium Sanitizer (200 ppm; EcoLab, St. Paul, MN).

Antimicrobial Treatments of Brine

Citric acid (CA, 0.1% and 0.2%), hydrogen peroxide (HP, 2000 ppm and 4000 ppm), and dimethyl dicarbonate (DMDC, 250 ppm and 500 ppm) were used to treat spent brine. To produce comparable results, the same concentrations of each antimicrobial that were used for fresh brine were used for spent brine. Like UV treatment, 50 mL sample of brine was taken before inoculation to check for pH, salt concentration, and background microflora. The brine was then inoculated with approximately 6.0 log CFU/mL *L. monocytogenes* cocktail and circulated for a minute to mix. A specific concentration of an antimicrobial was then added and allowed to mix for about a minute. A sample was taken that was named zero-minute sample and brine was circulated with added antimicrobial for 120 minutes. Samples were withdrawn at regular intervals.

Combination of UV and Antimicrobial Treatments of Brine

Brine was also treated with combination of UV with each antimicrobial. Once the brine was inoculated with approximately 6.0 log CFU/mL *L. monocytogenes* cocktail and antimicrobial was added as described above (section: Antimicrobial Treatment), a zero minute sample was taken. Immediately after that the UV was turned on. Brine was exposed to UV and antimicrobial simultaneously for 120 minutes and samples (50 mL) were withdrawn at regular intervals.

Microbiological analysis

Samples of test brines were serially diluted using 0.1% peptone and plated on to duplicate MOX agar plates followed by 48 hours incubation at 35°C. Typical black colonies were

considered as presumptive for *L. monocytogenes*. After incubation, colonies were counted and results were reported in CFU/mL. An enrichment procedure was also performed to detect the cells once population reach to detection limit (1.00 log CFU/mL) and are no longer recoverable by direct plating. For this purpose, 50 mL Brain Heart Infusion (BHI; Difco, Becton Dickinson and Company, Franklin Lakes, NJ) broth was inoculated with the 10 mL brine sample followed by 48 hours incubation at 35°C. After 24 hours, a loop-full of culture from BHI was streaked on MOX plates followed by 48 hours incubation at 35°C. Then, results were reported in presence or absence of *L. monocytogenes* in the samples. Typical colonies on MOX were confirmed using *Listeria* API.

Statistical Analysis

Each experiment of UV, antimicrobials, and combination of UV and each antimicrobial were replicated three times. Results of direct plating generated quantitative data that were analyzed using JMP statistical software version 6.00 (SAS Institute; Cary NC). Using regression analysis of these data, rates of *L. monocytogenes* reduction in brines were calculated for each treatment. Since the regression analysis was performed on each replication of all the treatments, there are more than one statistical models of this study. An example of these models for one of the treatments used in spent brine is described in detail in Appendix C. The mean rate of reduction was then obtained using one way ANOVA, where the value of α was used as 0.05. The results of enrichment studies were qualitative that described either presence or absence of *L. monocytogenes* in samples. Therefore, they were used to illustrate Time (in minutes) to reach a non-detectable level in brines, and therefore, the total processing time.

RESULTS

The results of total aerobic plate count showed that population of background micro-flora (aerobic mesophiles) in brine ranged from 1.9 to 4.3 log CFU/mL in different batches. The average pH of brine was on the acidic side, 5.5, with an average salt concentration of 15.3%. An average of 171.0 mg/L total solids and 1.0 mg/L total suspended solids were present in brine.

Microbiological Analysis of Brine

Figures 5.2, 5.3, and 5.4 explain the inactivation trend of *L. monocytogenes* in response to all the treatments. Table 5.2 provides the comparison of rate of reduction of *L. monocytogenes* and total time required (for each treatment) to reduce the number of this pathogen to a non-detectable level through enrichment.

An average starting *L. monocytogenes* population for all treatments was 5.8 log CFU/mL of brine. The slopes of all treatments were negative, which indicates the reduction in population with time. When brine was treated with UV alone, the population of *L. monocytogenes* reduced from 6.0 log CFU/mL to below the detection limit (i.e., 1 log CFU/mL) in 45 minutes (Figure 5.2) with a reduction rate of 0.12 log CFU/mL per minute (Table 5.2). However, *L. monocytogenes* population was detectable by enrichment throughout the 120 minute time-period.

When brine was treated with UV+4000 ppm HP and UV+2000 ppm HP, the *L. monocytogenes* population decreased from 5.8 log CFU/mL to below the detection limit in 45 minutes (Figure 5.2) with the reduction rate of 0.12 log CFU/mL per minute (Table 5.2). Moreover, the results of enrichment studies showed that the treatments UV+4000 ppm HP and UV+2000 ppm HP required 120 minutes to reach a non-detectable level of *L. monocytogenes*, whereas all other treatments and control required more than 120 minutes.

In general, it was observed that the reduction rate values of a few treatments were not significantly different than each other. Such treatments can, therefore, be grouped together and given the same rank. The final ranking, in decreasing order of reduction rate, is as follows: (i) UV+500 ppm DMDC, UV+4000 ppm HP, UV+2000 ppm HP, UV alone, UV+250 ppm DMDC, UV+0.5% CA, and UV+0.2% CA; and (ii) 250 ppm DMDC and 500 ppm DMDC, 4000 ppm HP, 2000 ppm HP, 0.2% CA, 0.5% CA, and Control. Based on the time (in minutes) required to reach a non-detectable level of *L. monocytogenes*, the inactivation of *L. monocytogenes* followed the order: (i) UV+4000 ppm HP and UV+2000 ppm HP, and; (ii) all other treatments. The treatments that required more than 120 minutes to reach a non-detectable level were ranked same since the brine was processed for 120 minutes.

DISCUSSION

Brining is one of the prime post-processing areas in food manufacturing where food contamination is very likely to occur. Moreover, organisms such as *L. monocytogenes* that can survive at high salt concentration and low temperature are of a major concern due to its low infective dose and high fatality rate. Thus, it is very important to process brine particularly when it is recycled to maintain zero tolerance policy of this pathogen in ready-to-eat food products. USDA observed that most of the recent recalls of *L. monocytogenes* are associated primarily with various fully cooked chicken products, frozen egg rolls, ready-to-eat meat products, and sausage products. Because brine is used to quickly cool thermally processed RTE products, it is a potential point in the process where *L. monocytogenes* contamination may occur. Not much work has been done on processing of brine to reduce or remove this pathogen. This study focuses on the use of the "multiple hurdle technology" to reduce the *L. monocytogenes* population in spent brine. Specifically, several combinations of UV and antimicrobials, such as citric acid, dimethyl dicarbonate, and hydrogen peroxide, were used in this study.

This research showed that when spent brine was treated with UV alone it required 45 minutes to achieve around 5.0 log CFU/mL reduction in the population of *L. monocytogenes*. This result does not corroborate well with the fresh brine study *(19)* where UV alone reduced around 5.0 log CFU/mL listeriae population in less than 15 minutes. This may be due to the presence of organic matter in spent brine. It is known that the penetration ability of UV light through liquids is poor, with the exception of clear water *(21)*. As the penetration of UV light into juices is about 1 mm for absorption of 90% of the light, in general, it is recommended to have a turbulent flow of liquid during UV processing *(15)*. The penetration ability of UV light

depends on the type of liquid, its UV absorption capacity, soluble solids, and suspended matter present in the liquid. Moreover, a large amount of soluble solids implies lower intensity of UV penetration in the liquid. Large suspended particles may also block the incidence of light on the microbial load *(5, 21)*. In a critical review on existing and potential application of UV in food industry, Bintsis et al. *(5)* have noted that in distilled water UV radiation at 254 nm would suffer 30% loss in intensity 40 cm below the surface, while sea water would suffer the same loss over approximately 10 cm. Therefore, a lower penetration of UV in spent brine resulted in lower reduction of *L. monocytogenes* population in spent brine compared to fresh brine in a relatively short time.

On the whole, a combination of UV and HP was found to be the most effective (based on time required to reduce the population to non-detectable level) in spent brine. This finding is in accordance with Larson et al. (1999) who also observed that *L. monocytogenes* survival was inhibited by ≥ 0.02% HP in recycled cheese brine (with 22.2% salt concentration at pH value of 5.3) *(17)*. In contrast, the same combination of UV and HP was observed relatively less effective in fresh brine *(19)*. This explains the manner in which changes in pH affect the antimicrobial activity of HP. The average pH of spent brine, 5.5, was on the acidic side. Baldry *(3)* studied the effect of pH on the antimicrobial activity of HP. He showed that 5 ppm HP inhibits the growth of *Pseudomonas aeruginosa* at pH 5.0, but 10 ppm is required at pH 6.7, and 50 ppm is required at pH 8.0. This study also showed that 3% solution of HP killed *Bacillus subtilis* spores in 3 hours at pH 5.0. However, the same solution required 6 hours at pH 6.5 and pH 8.0. Thus, HP shows better antimicrobial activity in acidic pH and slightly lower activity in neutral pH. However, HP's overall effectiveness is diminished at alkaline pH *(12)*. Thus, lower pH of spent

brine (5.5) than fresh brine (7.1) may have exhibited a positive effect on antimicrobial property of HP.

Combination of UV with 0.5% CA was not so effective with spent brine (Table 5.2). We conjecture that this phenomenon was observed because of the presence of organic matter in spent brine. As mentioned earlier, organic matter hinders UV penetration through spent brine. Consequently, cells do not receive adequate UV dose, which results in longer processing time to achieve the same log reduction. Moreover, we also expect some proteins to be present in spent brine since it was used to process frankfurters. These proteins may have a buffering capacity and so the same concentration of citric acid would not be able to reduce the pH of spent brine as low as it would in clean brine. It was observed that the average pH of fresh brine dropped from 7.1 to 2.1 by both 0.5% and 0.2% CA (data not shown). In contrast, the average pH of spent brine dropped from 5.5 to 3.6 and 4.1 through the addition of 0.5% and 0.2% CA, respectively. Thus, the combination of lower UV penetration and pH reduction may have reduced the effectiveness of UV+0.5% CA treatment in spent brine.

In spent brine the reduction rate achieved by UV+500 ppm DMDC was not significantly different from the UV+4000 ppm HP (Table 5.2). However, the former treatment needed more than 120 minutes to reach a non-detectable level. Williams et al. (2005) showed that the combination of ozone with DMDC achieved greater reduction in the population of *E. coli* O157:H7 and *Salmonella* in apple cider compared to orange juice. They reasoned that more organic matter was present in orange juice than apple cider since the juice was home-style with added pulp *(26)*. We believe that organic matter present in spent brine reduced the availability of DMDC for microbial interaction and also reduced the UV penetration. This resulted in lower *L.*

monocytogenes reduction rate and higher treatment time to reach a non-detectable level in spent brine.

Our research showed that, in general, all treatments were more effective in reducing the population of listeriae in fresh brine compared to spent brine. This was because the spent brine contained a lot of organic matter that hindered UV penetration. Moreover, organic matter is also thought to have a quenching effect on antimicrobials and thus reduced their availability for interaction with microorganisms. Therefore, the combined effect of low UV penetration and less availability of antimicrobials resulted in long processing times of spent brine with the concentrations of antimicrobials used in this study. However, we conjecture that an increase in the concentration of these antimicrobials with UV may effectively reduce the *L. monocytogenes* population in less time.

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Table 5.2: *Listeria monocytogenes* Inactivation during Ultraviolet Light Treatment of **Used/Spent Brines** Containing Citric Acid (0.2 and 0.5%), Dimethyl Dicarbonate (250 and 500ppm), and Hydrogen Peroxide (2000 and 4000ppm) in a Recirculating Brine Chiller System

Treatment	Mean Rate of Reduction (Log CFU/mL per minute)	Average R^2	Time (min.) to Reach ND*
2000 ppm HP Control	5.7×10^{-4} [a]	0.24	>120
0.5% CA Control	0.001 [a]	0.31	>120
0.2% CA Control	0.002 [a]	0.96	>120
UV Negative Control	0.002 [a]	0.44	>120
4000 ppm HP Control	0.004 [a]	0.48	>120
500 ppm DMDC Control	0.02 [a]	0.94	>120
250 ppm DMDC Control	0.05 [a,b]	0.92	>120
UV + 0.2% CA	0.09 [b,c]	0.96	>120
UV + 0.5% CA	0.11 [b,c]	0.97	>120
UV+250 ppm DMDC	0.11 [b,c]	0.99	>120
UV Positive Control	0.12 [c]	0.94	>120
UV+2000 ppm HP	0.12 [c]	0.97	120
UV+4000 ppm HP	0.12 [c]	0.94	120
UV+500 ppm DMDC	0.13 [c]	0.93	>120

Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD.

*ND = Non Detectable level; the organism did not recover through enrichment.

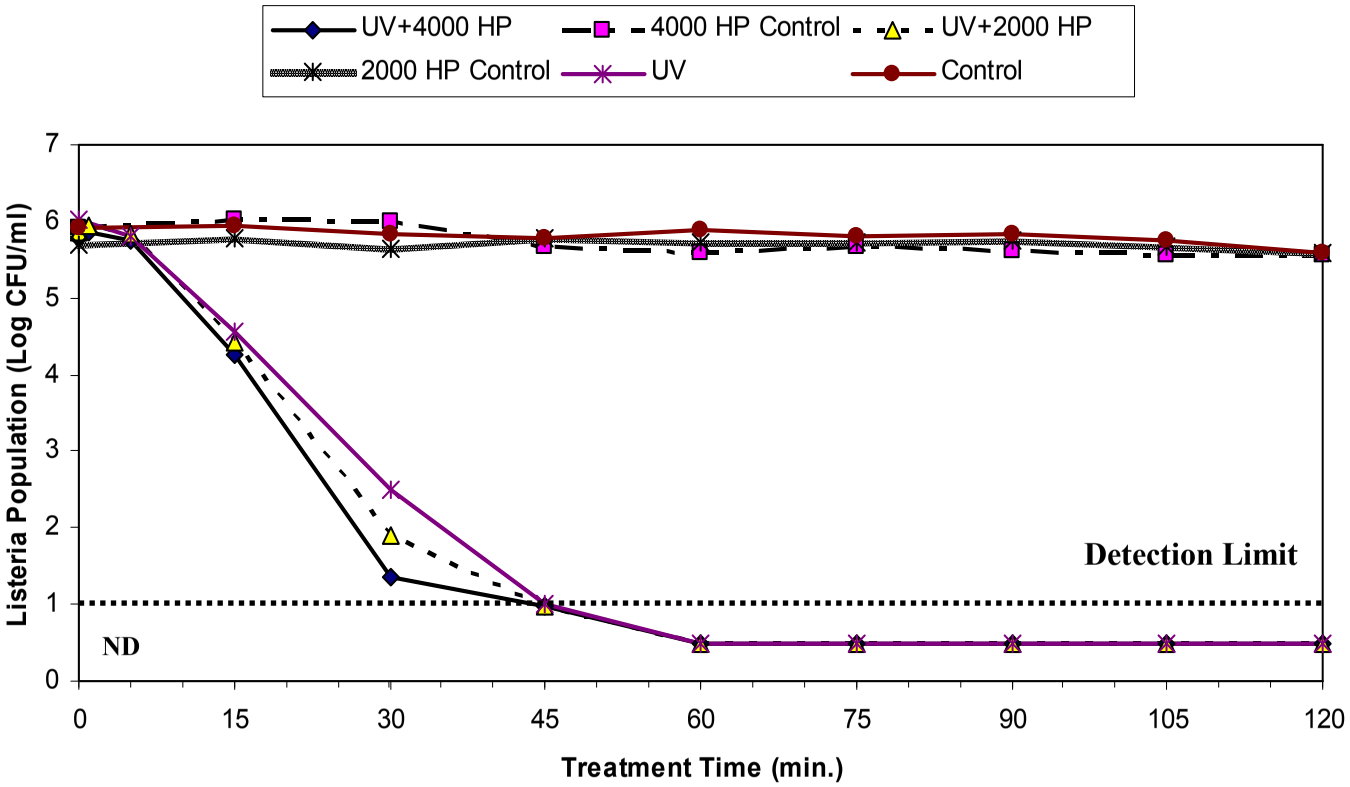


Figure 5.2: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with HP (2000 and 4000 ppm) treatments in Spent Brine. ND = Not Detected; the organism did not recover through direct surface plating.

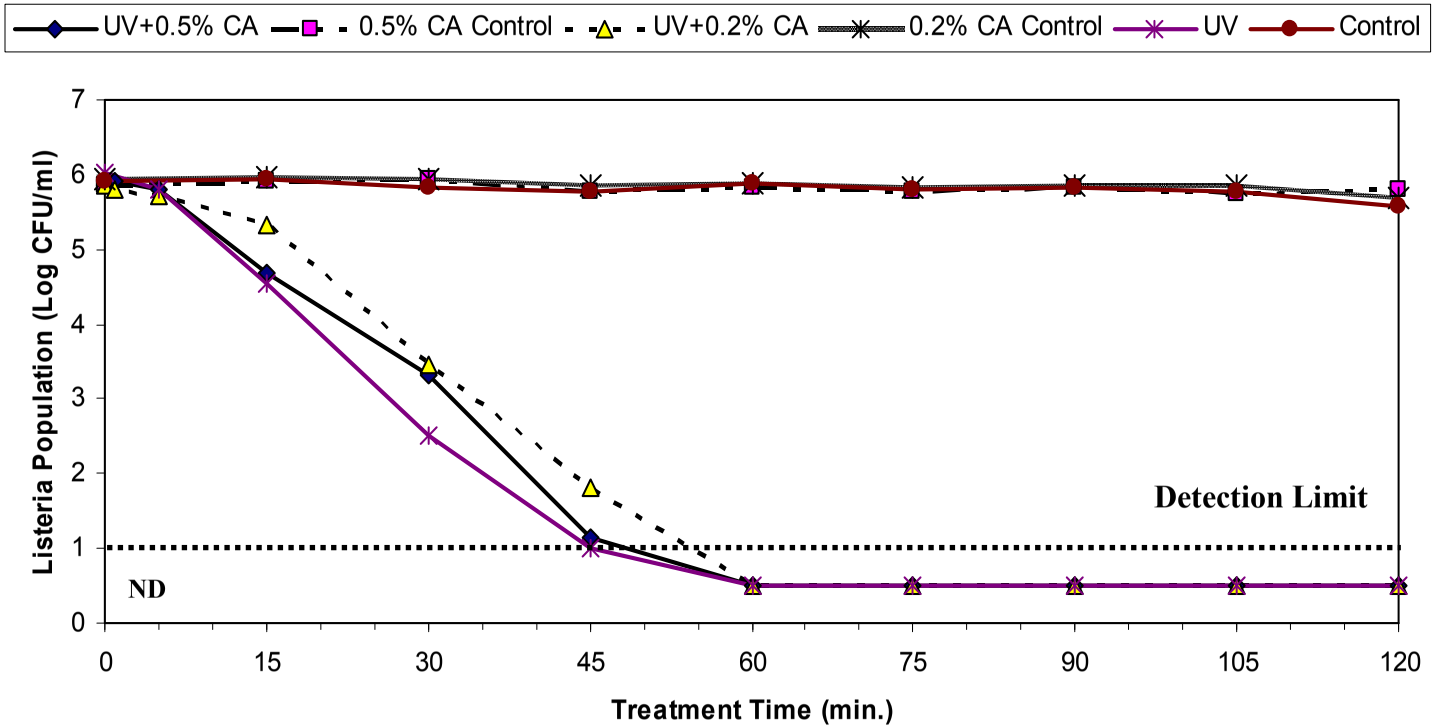


Figure 5.3: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with CA (0.2 and 0.5%) treatments in Spent Brine. ND = Not Detected; the organism did not recover through direct surface plating.

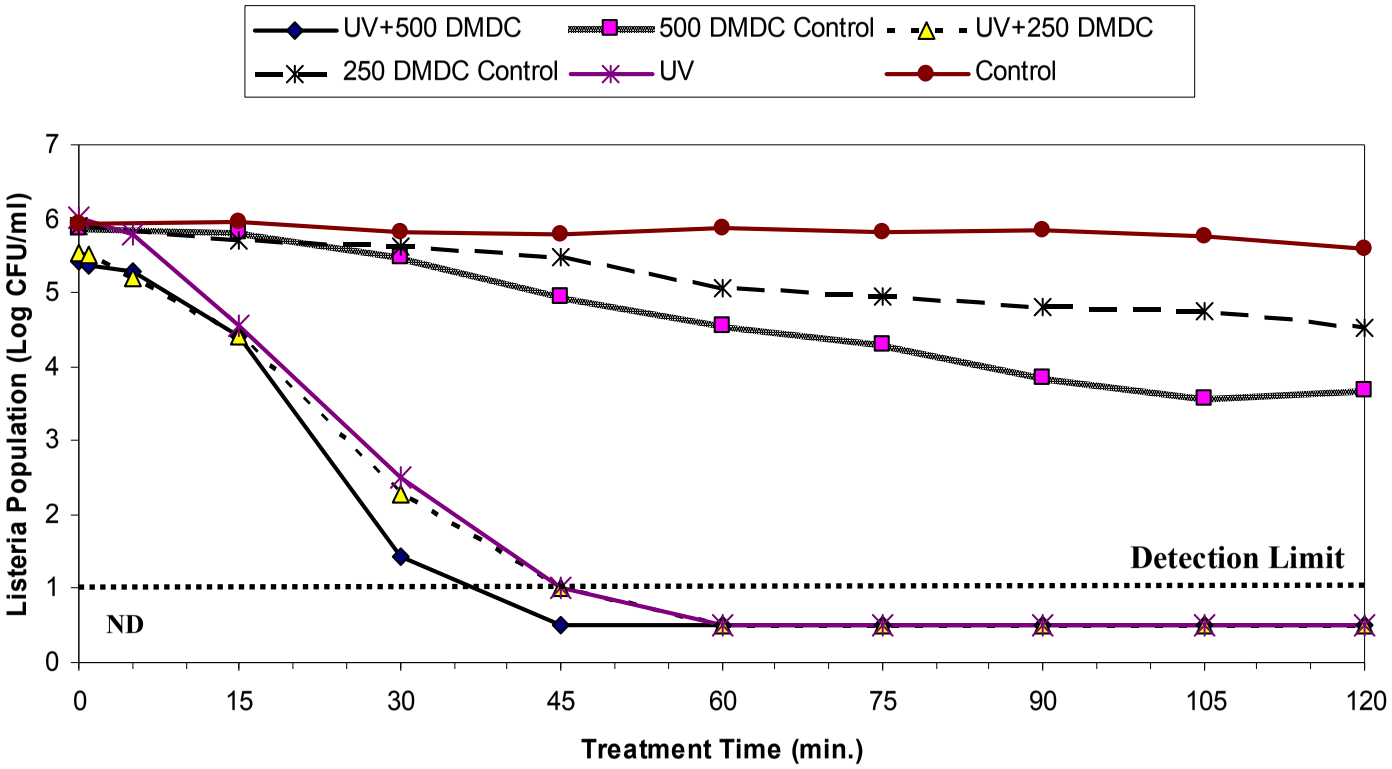


Figure 5.4: Inactivation of *Listeria monocytogenes* through UV alone and combination of UV with DMDC (250 and 500 ppm) treatments in Spent Brine. ND = Not Detected; the organism did not recover through direct surface plating.

CONCLUSION

Brining is one of the prime post-processing areas in food manufacturing where food contamination is very likely to occur. Moreover, organisms such as *L. monocytogenes* that can survive at high salt concentration and low temperature are of a major concern due to its low infective dose and high fatality rate. Thus, it is very important to process brine particularly when it is recycled. Based on "multiple hurdle technology" principle, we processed fresh/unused and spent/used chill brines using several combinations of UV and antimicrobials, such as citric acid, dimethyl dicarbonate, and hydrogen peroxide.

Results of fresh brine studies showed that the combination of UV and 500 ppm DMDC had the highest rate of decline and required a total time of 60 minutes to reduce the population to a non-detectable level through enrichment. In contrast, UV+0.5% CA had significantly lower reduction rate than UV+500 ppm DMDC, but was the most effective treatment in terms of complete destruction of listeriae cells; i.e., cells could not be recovered after 45 minutes even through enrichment. Overall, a combination of treatments was more effective than any of the treatments alone except 0.5% CA control, which was almost as effective as UV+0.5% CA. The lowest processing time to reduce the number of *Listeria* cells to a non-detectable level via enrichment was 45 minutes, which was obtained with UV+0.5% CA treatment. Both UV+500 ppm DMDC and 0.5% CA control required 60 minutes to achieve the same result.

Results of spent brine studies showed that the combination of UV with 2000 and 4000 ppm HP, which was one of the least effective treatments in fresh brine, was the most effective treatment. These treatments obtained the highest rate of decline, which was however lower than the highest rate of decline obtained in fresh brine with the treatment UV+500 ppm DMDC. The lowest processing time of brine to reduce the listeriae population to a non-detectable level

through enrichment was 120 minutes in spent brine, which was much higher than 45 minutes when compared to fresh brine.

Our research showed that, in general, all the treatments were more effective in reducing the population of listeriae in fresh brine than spent brine. This was because the spent brine contained a lot of organic matter that hindered in UV penetration. Moreover, organic matter is also thought to have a quenching effect on antimicrobials and thus reduced their availability for interaction with microorganisms. Therefore, the combined effect of low UV penetration and less availability of antimicrobials resulted in long processing times of spent brine with the tested concentration of antimicrobials. However, we conjecture that an increase in the concentration of these antimicrobials with UV may effectively reduce the *L. monocytogenes* population in less time.

FUTURE WORK

Brine is very likely to get contaminated with foodborne pathogens, such as *L. monocytogenes*, if it is established in plant already. Contaminated brine can pose serious threat of listeriosis outbreak since this pathogen can survive well in brine. Even though this is an important area in food safety especially of RTE foods, there is a lack of work in this area. Thus, following are the main field of research that will enhance our knowledge and may improve current practices of brine processing in industry.

1. Our work showed that high concentration of organic matter and solids present in spent brine negatively affect the effectiveness of UV and antimicrobials. One approach to solve this problem would be filtration of spent brine prior to any treatment. Filtration will reduce the concentration of solids and other particles present in the spent brine and may improve the effectiveness of UV and antimicrobials.
2. Efficacy of other non-thermal processes, such as high pressure, pulsed electric field, should also be determined alone and in combination with antimicrobials or other processes to process brine. High pressure processing has been shown as an effective mean to reduce the population of listeriae cells in various media such as buffer with pH value 7.0, liquid whole egg (pH 8.0), and oysters. Moreover, addition of antimicrobial such as nicin, citric acid, and lactic acid along with high pressure found to be very effective at reduction of *L. monocytogenes* from foods. Thus, this treatment might be promising at reducing the population of listeriae in brine. Similarly pulsed electric field has also been found effecient at reducing the listeria population in milk *(47)*.
3. Bacteria adapt to any unfavorable environment that can lead to an increased pathogenisity of the organism; for example, antibiotic resistant *Salmonella*. UV has been used for inactivation

of various pathogens in wide variety of foods. Since UV affect the DNA of the cell, it might be possible that sub-lethally UV injured *L. monocytogenes* cells show higher virulence or greater resistance to other environmental conditions. Thus, it may be useful to study a behavior of UV stressed cells in presence of high salt, low pH, low temperature, etc.

4. UV can be effectively used for this purpose if the specific dosage requirement is known. Therefore, determination of UV dose to remove *L. monocytogenes* from spent brine is also important.

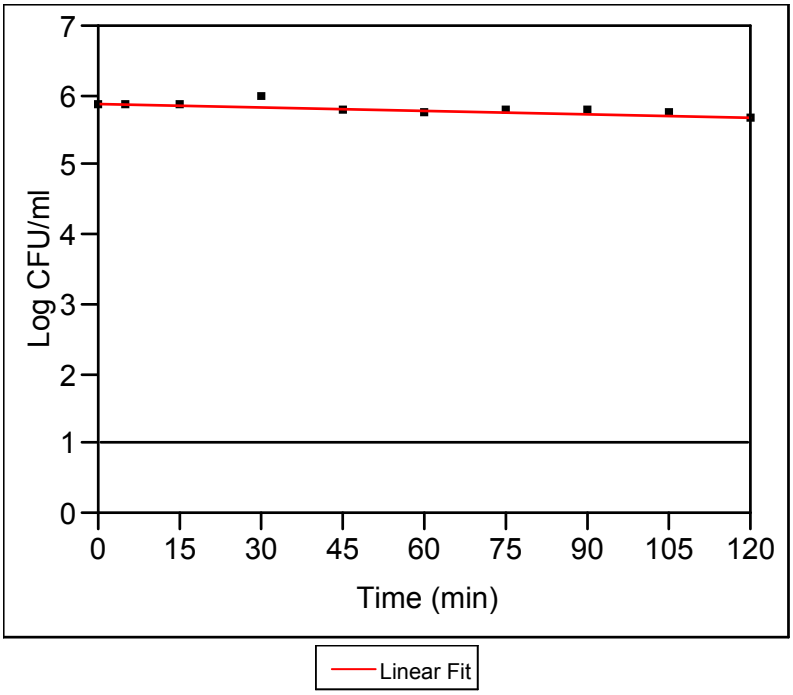
APPENDIX A: REGRESSION ANALYSES

As mentioned in the Materials and Methods section of Chapter IV, for each treatment, we conducted three runs, obtained the reduction rates of *L. monocytogenes* (i.e., slope value), and the R^2 values. We then calculated the mean of the three slope and R^2 values. The mean value of slope and mean R^2 values for each treatment are tabulated in Table 1 for fresh brine and Table 2 for spent brine.

This appendix briefly highlights the steps followed during the regression analysis for UV (one of the several treatments) used for fresh and spent brines in this research.

Regression Analysis for 3 replications of UV Negative Control in Fresh Brine

RUN=1



Regression Model (Linear Fit)

Log CFU/mL = 5.8611327 - 0.0014887 * Time

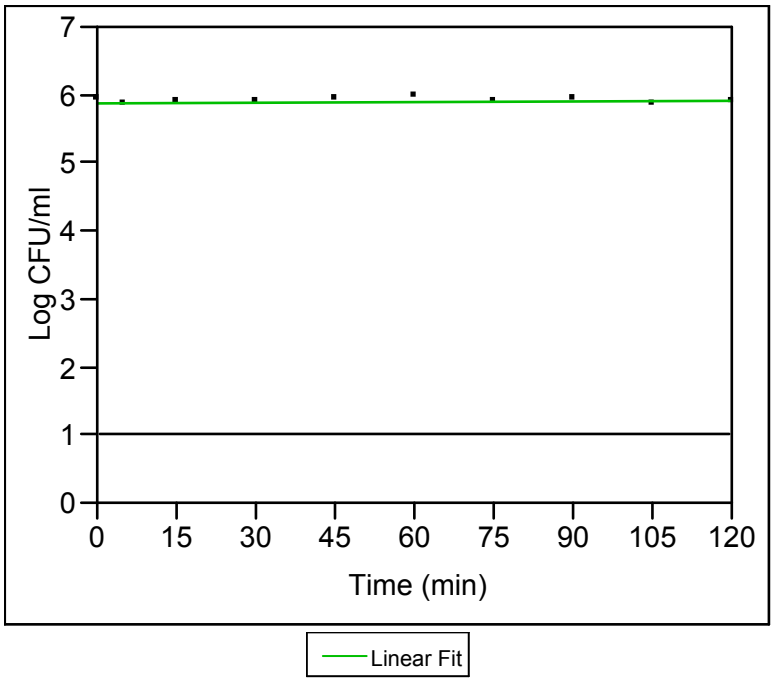
Summary of Fit

RSquare	0.609347
RSquare Adj	0.560515
Root Mean Square Error	0.053676
Mean of Response	5.78
Observations (or Sum Wgts)	10

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.03595146	0.035951	12.4785
Error	8	0.02304854	0.002881	Prob > F
C. Total	9	0.05900000		0.0077

RUN=2



Regression Model (Linear Fit)

Log CFU/mL = 5.8887748 + 5.9177e-5 * Time

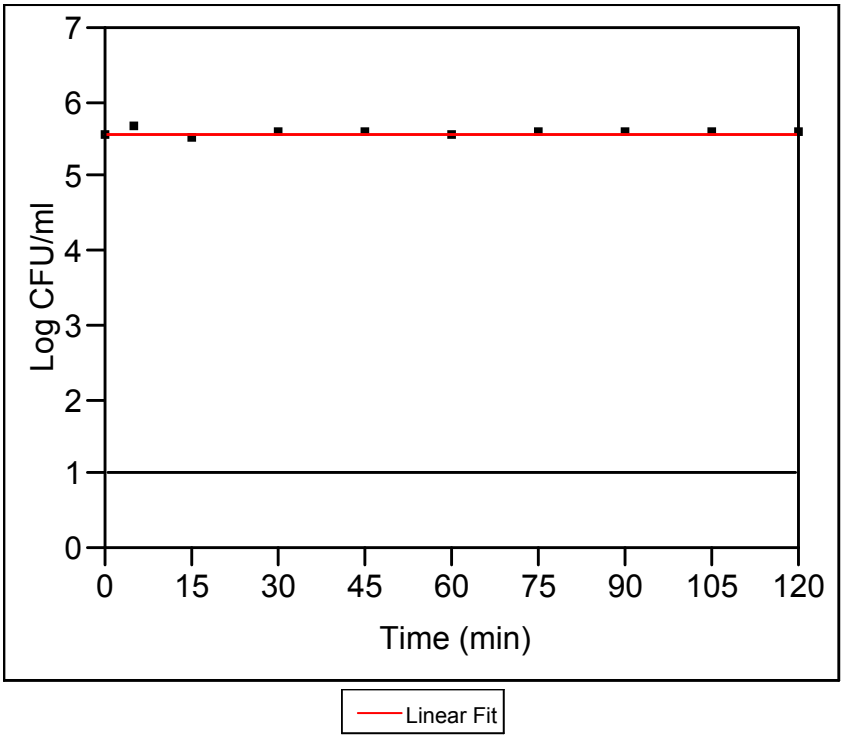
Summary of Fit

RSquare	0.005942
RSquare Adj	-0.11831
Root Mean Square Error	0.034466
Mean of Response	5.892
Observations (or Sum Wgts)	10

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.00005681	0.000057	0.0478
Error	8	0.00950319	0.001188	Prob > F
C. Total	9	0.00956000		0.8324

RUN=3



Regression Model (Linear Fit)

Log CFU/mL = 5.5466158 + 8.0444e-5 * Time

Summary of Fit

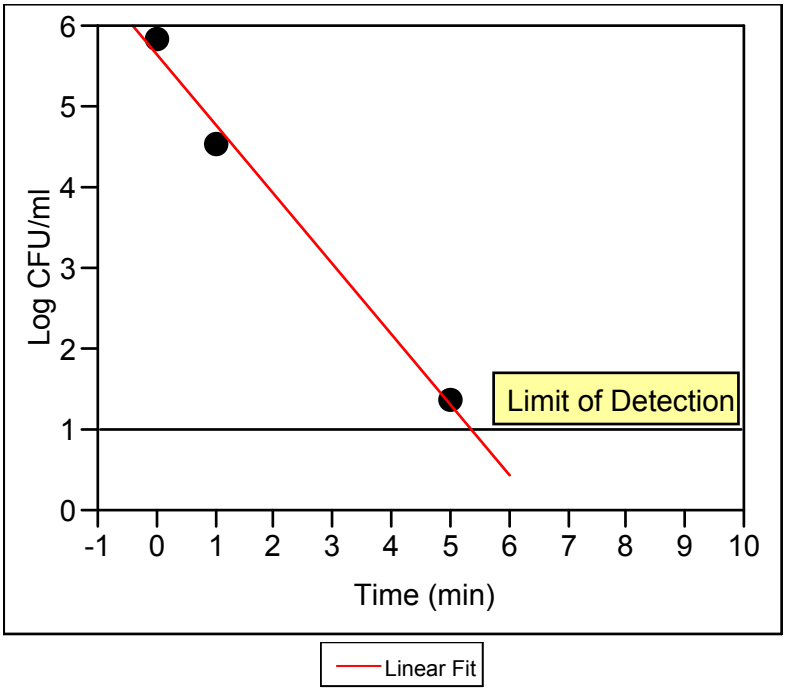
RSquare	0.007899
RSquare Adj	-0.11611
Root Mean Square Error	0.040597
Mean of Response	5.551
Observations (or Sum Wgts)	10

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.00010498	0.000105	0.0637
Error	8	0.01318502	0.001648	Prob > F
C. Total	9	0.01329000		0.8071

Regression Analysis for 3 replication of UV Positive Control in Fresh Brine

RUN=1



Regression Model (Linear Fit)

Log CFU/mL = 5.6442857 - 0.8671429 * Time

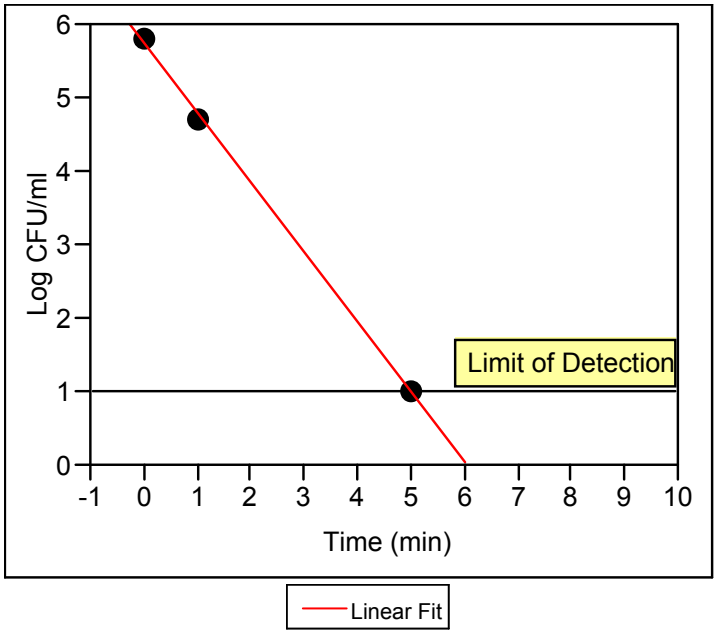
Summary of Fit

RSquare	0.989558
RSquare Adj	0.979116
Root Mean Square Error	0.333295
Mean of Response	3.91
Observations (or Sum Wgts)	3

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	10.527114	10.5271	94.7657
Error	1	0.111086	0.1111	Prob > F
C. Total	2	10.638200		0.0652

RUN=2



Regression Model (Linear Fit)

Log CFU/mL = 5.7333333 - 0.95 * Time

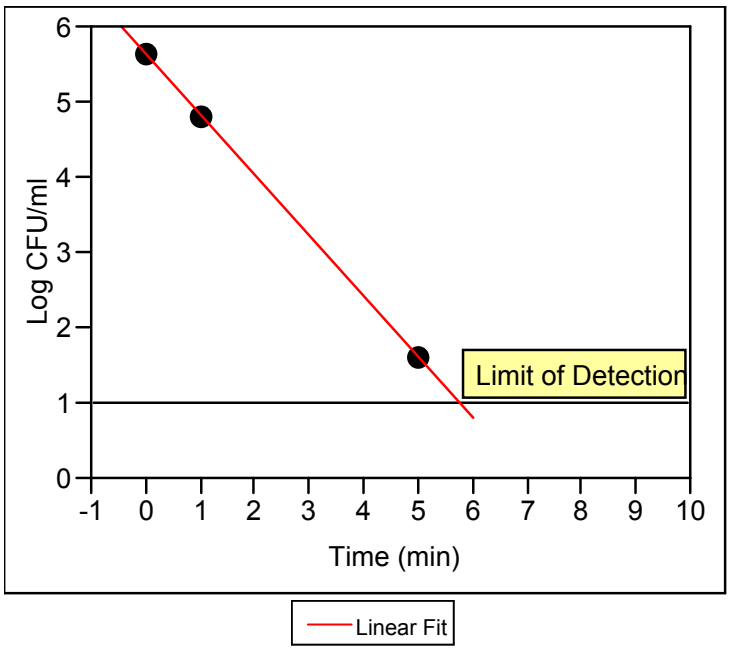
Summary of Fit

RSquare	0.999077
RSquare Adj	0.998155
Root Mean Square Error	0.108012
Mean of Response	3.833333
Observations (or Sum Wgts)	3

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	12.635000	12.6350	1083.000
Error	1	0.011667	0.0117	Prob > F
C. Total	2	12.646667		0.0193

RUN=3



Regression Model (Linear Fit)

Log CFU/mL = 5.62 - 0.805 * Time

Summary of Fit

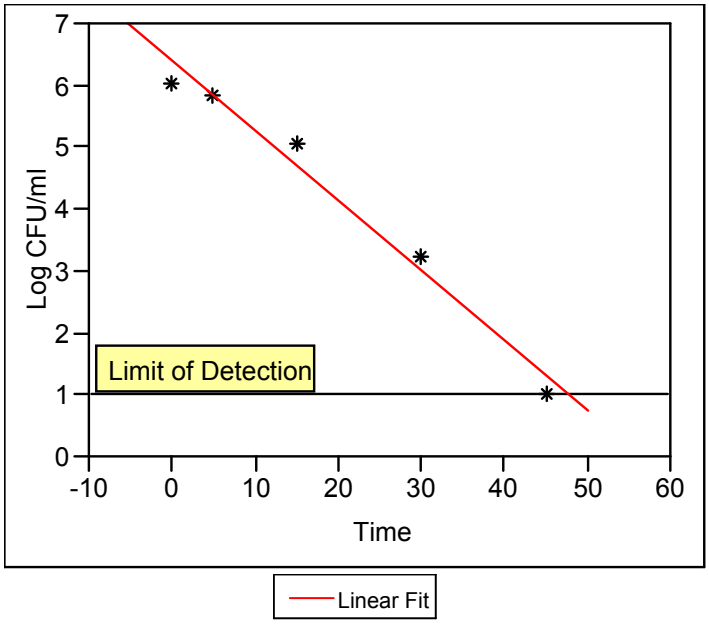
RSquare	0.999884
RSquare Adj	0.999769
Root Mean Square Error	0.032404
Mean of Response	4.01
Observations (or Sum Wgts)	3

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	9.0723500	9.07235	8640.333
Error	1	0.0010500	0.00105	Prob > F
C. Total	2	9.0734000		0.0068

Regression Analysis for 3 replication of UV Positive Control in Spent Brine

RUN=1



Regression Model (Linear Fit)

Log CFU/mL = 6.3845255 - 0.1130803 * Time

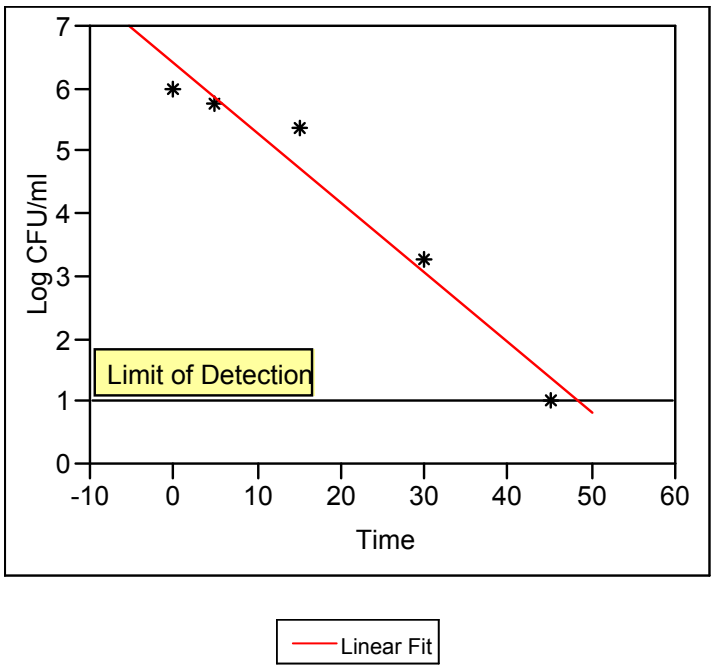
Summary of Fit

RSquare	0.976123
RSquare Adj	0.968164
Root Mean Square Error	0.377942
Mean of Response	4.236
Observations (or Sum Wgts)	5

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	17.518399	17.5184	122.6432
Error	3	0.428521	0.1428	Prob > F
C. Total	4	17.946920		0.0016

RUN=2



Regression Model (Linear Fit)

Log CFU/mL = 6.3935036 - 0.1118686 * Time

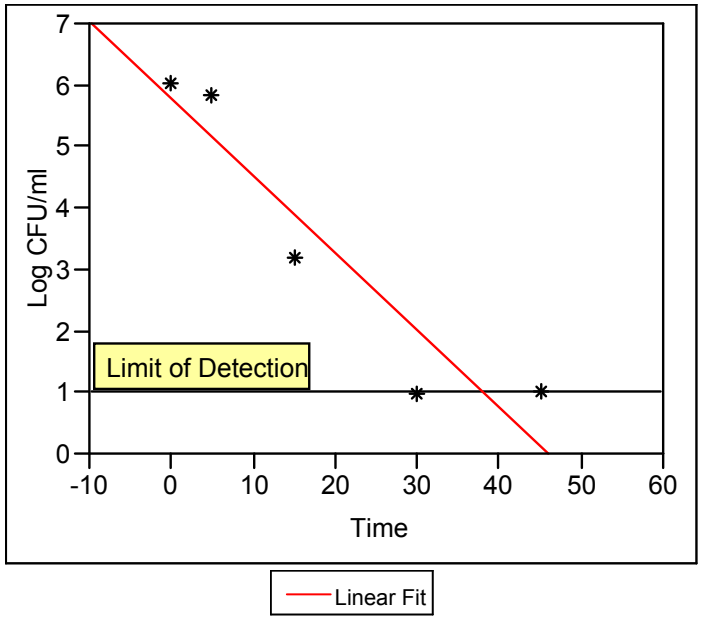
Summary of Fit

RSquare	0.956117
RSquare Adj	0.94149
Root Mean Square Error	0.512151
Mean of Response	4.268
Observations (or Sum Wgts)	5

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	17.144984	17.1450	65.3643
Error	3	0.786896	0.2623	Prob > F
C. Total	4	17.931880		0.0040

RUN=3



Regression Model (Linear Fit)

Log CFU/mL = 5.7974818 - 0.1256569 * Time

Summary of Fit

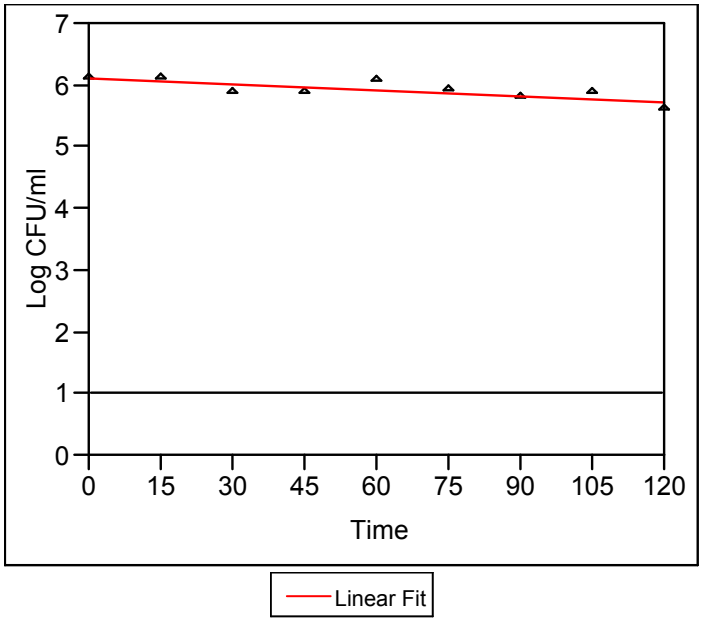
RSquare	0.885332
RSquare Adj	0.847109
Root Mean Square Error	0.966395
Mean of Response	3.41
Observations (or Sum Wgts)	5

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	21.631841	21.6318	23.1624
Error	3	2.801759	0.9339	Prob > F
C. Total	4	24.433600		0.0171

Regression Analysis for 3 replications of UV Negative Control in Spent Brine

RUN=1



Regression Model (Linear Fit)

Log CFU/mL = 6.1113333 - 0.0033556 * Time

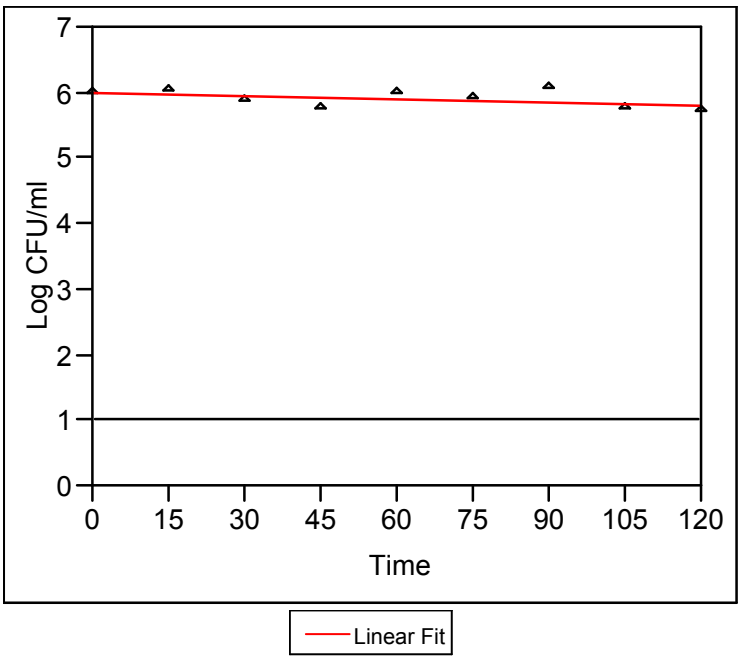
Summary of Fit

RSquare	0.683483
RSquare Adj	0.638267
Root Mean Square Error	0.100281
Mean of Response	5.91
Observations (or Sum Wgts)	9

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.15200667	0.152007	15.1157
Error	7	0.07039333	0.010056	Prob > F
C. Total	8	0.22240000		0.0060

RUN=2



Regression Model (Linear Fit)

Log CFU/mL = 5.9844444 - 0.0015 * Time

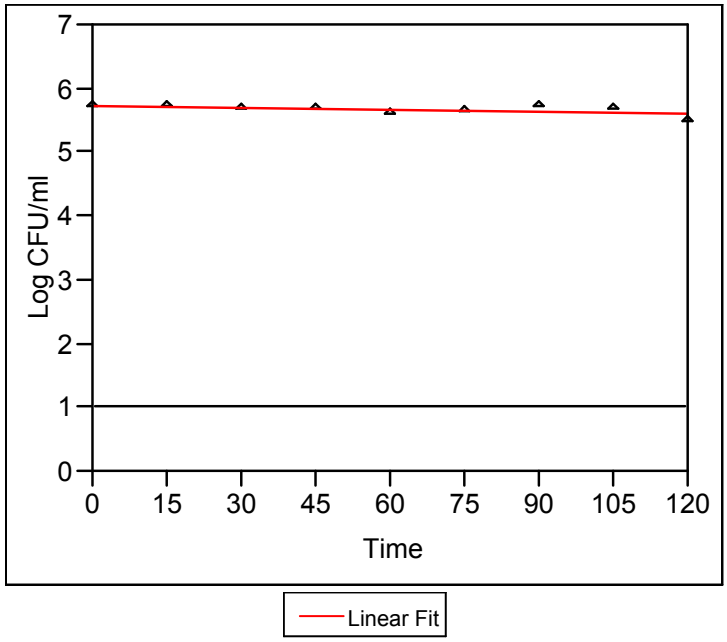
Summary of Fit

RSquare	0.237263
RSquare Adj	0.128301
Root Mean Square Error	0.118108
Mean of Response	5.894444
Observations (or Sum Wgts)	9

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.03037500	0.030375	2.1775
Error	7	0.09764722	0.013950	Prob > F
C. Total	8	0.12802222		0.1836

RUN=3



Regression Model (Linear Fit)

Log CFU/mL = 5.7155556 - 0.0011111 * Time

Summary of Fit

RSquare	0.40366
RSquare Adj	0.318468
Root Mean Square Error	0.059308
Mean of Response	5.648889
Observations (or Sum Wgts)	9

Analysis of Variance

Source	*DF*	*Sum of Squares*	*Mean Square*	*F Ratio*
Model	1	0.01666667	0.016667	4.7383
Error	7	0.02462222	0.003517	Prob > F
C. Total	8	0.04128889		0.0660

VITA

Priti Parikh was born and raised in Surat (Gujarat, India). In May 2002 she received the Masters of Science (M.Sc.) degree in Microbiology from Sardar Patel University, Anand (Gujarat, India). She worked as a Product Executive with Span Diagnostics Ltd. before joining the Department of Food Science and Technology at Virginia Tech. as a Ph.D. student in Spring 2005. Under the guidance of Robert C. Williams, Ph.D., she initiated research in the area of Food Microbiology, and completed this research in Fall 2007. She has presented her research at IAFP conference in 2006 and 2007. During her stay at Virginia Tech, Priti served as teaching and research assistants for the department, and led/participated in various national level product development competitions.