USE OF ULTRAVIOLET LIGHT FOR THE INACTIVATION OF
LISTERIA MONOCYTOGENES AND LACTIC ACID BACTERIA
SPECIES IN RECYCLED CHILL BRINES

Karol Marie Gailunas

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
In partial fulfillment of the requirements for the degree of

Master of Science
in
Food Science and Technology

Susan S. Sumner, Chair
Christine Z. Alvarado
Robert C. Williams

June 30, 2003
Blacksburg, VA

Key words: Listeria monocytogenes, lactic acid bacteria, ultraviolet light, brines

Copyright 2003, Karol M. Gailunas
USE OF ULTRAVIOLET LIGHT FOR THE INACTIVATION OF *LISTERIA MONOCYTOGENES* AND LACTIC ACID BACTERIA SPECIES IN RECYCLED CHILL BRINES

Karol M. Gailunas

(ABSTRACT)

Ready-to-eat meat products have been implicated in several foodborne listeriosis outbreaks. Microbial contamination of these products can occur after the product has been thermally processed and is being rapidly chilled using salt brines. The objective of this study was to determine the effect of ultraviolet irradiation on the inactivation of *Listeria monocytogenes* and lactic acid bacteria in a model brine chiller system. Two concentrations of brines (7.9%w/w or 13.2%w/w) were inoculated with a ~6.0 log$_{10}$ CFU/ml cocktail of *L. monocytogenes* or lactic acid bacteria and passed through the ultraviolet (UV) treatment system for 60 minutes. Three replications of each bacteria and brine combination were performed and resulted in at least a 4.5 log reduction in microbial numbers in all treated brines after exposure to ultraviolet light. Bacterial populations were significantly reduced after five minutes exposure to UV light in the model brine chiller as compared to the control, which received no UV light exposure (P<0.05). The maximum rate of inactivation for both microorganisms in treated brines occurred between minute 1 and 15 of ultraviolet exposure. Overall, results indicate that inline treatment of chill brines with ultraviolet light (UVC) shows promise in inactivating *L. monocytogenes* and lactic acid bacteria. Due to the low capital involved in initiating a continuous inline UV system, the use of ultraviolet energy may prove to be beneficial for effectively controlling pathogens in recycled chill brines without interrupting the chilling operation. An inline ultraviolet system could be used in a hazard analysis and critical control points plan.
ACKNOWLEDGEMENTS

I would first like to thank my graduate advisor Dr. Susan Sumner for all of the guidance and direction she has given me throughout these past two years. She has been a mentor, teacher and a friend and she believed in me even when I didn’t believe in myself. I feel honored to have had this opportunity to work with her. I would also like to thank my committee members, Dr. Christine Alvarado and Dr. Robert Williams, for always having an open door for answering my questions and offering ideas. Additionally, I’d like to thank Dave Jones and Wafa Birbari at the Sara Lee Corporation for an endless amount of input and thoughts that helped give life to this project.

I would also like to thank my fiancé and best friend, Brent Quinn for being a hand to hold and a source of never-ending encouragement. He has been a foundation of strength for me during two difficult years apart from each other. I am very grateful for his sense of humor in which he has always been able to make me laugh, even when I thought I’d never be done with school. Thank you for always being there for me.

I’d like to offer a very special thank you to my family for their love and support including my parents Charles and Barbara Gailunas and siblings, Kate, Lynn, Sarah, Ali and Charlie. You have each helped and guided me through this in your own way. I am so lucky to have such amazing people in my life. Thank you to the one friend who’s known me the longest, Liz Flaherty. Thank you for being such a beautiful person, always lending an ear when I needed to talk, a shoulder when I needed to cry, and a smile when I needed a friend.

I’d also like to thank Daniel Schu and Bridget Meadows for the many hours spent helping me in the cooler and in the lab. I would also like to thank the very knowledgeable and cooperative departmental staff that is always available. Specifically I’d like to thank Wes Shilling, Brian Yaun, Walter Hartman, and Brian Smith.
Finally I would like to thank all the graduate students in the department for creating a fun environment to work in. I would especially like to thank Emily Hodson, for the good times, always taking a break with me, and the great friendship that we have built. These two years in school have developed into a lifelong friendship. Also, thanks to Renee Raiden, Valerie Gorsuch, Christine Piotrowski, Angie Hartman, Gabe Sanglay, Lindsay Millard, Michael Bazaco, and Megan Hereford for being friends as well as officemates.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS v
LIST OF TABLES vii
LIST OF FIGURES viii
INTRODUCTION 1

REVIEW OF LITERATURE 4
A. Listeria monocytogenes 4
1. Characteristics 4
2. Illness 4
3. Foodborne outbreaks 5
4. Factors affecting growth and survival 6
   a. Temperature 6
   b. Salt Concentration 7
   c. Water Activity 8
   d. pH 9
   e. Microbial competition 10
B. Lactic Acid Bacteria 12
1. Characteristics 12
2. Shelf-life and spoilage 13
3. Carnobacterium gallinarum 14
4. Enterococcus faecalis 15
5. Lactobacillus plantarum 16
C. Recycled Chill Brines 17
1. General 17
2. Survival of L. monocytogenes in brine 18
D. Ultraviolet radiation 19
1. General 19
2. Susceptibility of microorganisms 19
3. Efficacy and application of UV 20
4. Applications 20
5. Ultraviolet inactivation of L. monocytogenes 21
E. Conclusions 22

REFERENCES 24

USE OF ULTRAVIOLET LIGHT FOR THE INACTIVATION OF
LISTERIA MONOCYTOGENES AND LACTIC ACID BACTERIA
SPECIES IN RECYCLED CHILL BRINES 33

INTRODUCTION 34
MATERIALS AND METHODS 36
A. Brine preparation 36
B. Inoculum preparation 36
   1. *Listeria monocytogenes* 36
   2. Lactic Acid Bacteria 37
C. Survival Studies 38
D. Ultraviolet Treatment System 38
E. Microbial analysis 39
   1. *Listeria monocytogenes* 40
   2. Lactic Acid Bacteria 40
F. Statistical analysis 41

RESULTS AND DISCUSSION 42
CONCLUSIONS 47
REFERENCES 57
AREAS FOR FURTHER RESEARCH 59
APPENDIX I 60
APPENDIX II 61
APPENDIX III 62
VITAE 63
LIST OF TABLES

REVIEW OF LITERATURE

Table 1: USDA regulations for recycled chill brines, pg. 18

RESULTS AND DISCUSSION

Table 1: Bacterial populations of \textit{L. monocytogenes} (LM) plated on Modified Oxford Agar (MOX), pg. 48

Table 2: Bacterial populations of \textit{L. monocytogenes} (LM) plated on Tryptic Soy Agar supplemented with 0.6\% Yeast Extract (TSAYE), pg. 49

Table 3: Bacterial populations of lactic acid bacteria (LAB) plated on All Purpose Tween Agar supplemented with 0.0032\%w/v bromcresol purple dye (APT+BCP), pg. 50
LIST OF FIGURES

Figure 1: Mean microbial populations of brine inoculated with a six-strain mixture of *Listeria monocytogenes* plated on Modified Oxford Agar (MOX) after 1 hour in UV Treatment System, pg. 51

Figure 2: Mean microbial populations of brine inoculated with a six-strain mixture of *Listeria monocytogenes* plated on Tryptic Soy Agar (TSA) supplemented with 0.6% Yeast Extract (YE) after 1 hour in UV Treatment System, pg. 52

Figure 3: Mean microbial populations of brine inoculated with a three-strain mixture of lactic acid bacteria plated on All Purpose Tween (APT) Agar supplemented with 0.0032% (w/v) bromcresol purple dye (BCP) after 1 hour in UV Treatment System, pg. 53

Figure 4: Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 4°C over a 6 day period, pg. 54

Figure 5: Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 25°C over a 6 day period, pg. 55

Figure 6: Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 32°C over a 6 day period, pg. 56
INTRODUCTION

*Listeria monocytogenes* is a pathogen that poses a serious threat to public health. A major cause of human listeriosis is due to consumer exposure to *L. monocytogenes* from ready-to-eat meat products such as frankfurters and foods purchased at a deli counter. The food industry in the United States is currently under a “zero tolerance” policy for *L. monocytogenes* in ready-to-eat foods by both United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) and the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN), because the infective dose is unknown. Reduction in illness has eluded industry’s food safety efforts because *L. monocytogenes* is commonly found in the environment and is more resistant than most bacteria to conditions and treatments used to control foodborne pathogens. The organism’s optimum growth temperature is 30-37°C; however, it is known to grow at temperatures as low as 1°C and as high as 45°C (49). The ability of *L. monocytogenes* to grow at refrigeration temperatures, while competing organisms cannot, offers a competitive advantage for the microorganism. The growth of the organism on refrigerated, ready-to-eat food products causes a serious potential food safety hazard. Although *L. monocytogenes* can be destroyed if heated to a high enough temperature, there may be contamination of the food product after it has been thermally processed (93). Ready-to-eat meat products that have received heat treatment followed by cooling in brine before packaging may supply a more favorable environment for multiplication of *L. monocytogenes* because of the decrease in competitive microflora and the high salt tolerance of the organism (85). Due to the risk of cross-contamination, post-processing treatments are needed to inhibit growth of *L. monocytogenes* on ready-to-eat food products, such as frankfurters.

Frankfurters have been linked recently to several outbreaks, and sometimes frankfurters are eaten without reheating to a sufficient temperature (20). Additionally, due to the ubiquitous nature of *L. monocytogenes*, there are many possible modes of entry in processing facilities. Entry of *L. monocytogenes* into food processing plants occurs through soil on worker’s shoes and clothing and on transport equipment, animals which excrete the bacterium, raw foods or animal origin, and possibly healthy human carriers.
Once on frankfurters, the frankfurters provide an adequate pH, water activity ($a_w$), and nutrients for growth of the organism. A study by Samelis and Metaxopoulos (76) determined the major sources and routes of contamination of *Listeria* spp. in a meat processing plant. *Listeria monocytogenes* and other *Listeria* spp. were isolated from 51% and 49% of samples of frozen raw meat taken from several incoming lots. As a consequence, listeriae colonized certain processing sites where raw materials were handled and hygienic conditions were not strictly followed. Tumbled meats were contaminated heavily during tumbling as the need to operate tumblers continuously did not enable their proper cleaning and disinfection. Also the use of mechanically deboned turkey-neck meat in cooked sausages raised contamination at a pre-cooking stage (76). Another source of contamination on frankfurters by *L. monocytogenes* occurs through post process contamination during the cooling step when the frankfurters are sprayed with salt brines before packaging (62).

Recycled brine is frequently used in food processing plants to cool thermally processed products. The rapid cooling of the product minimizes the chance that the product will be exposed to temperatures that permit bacterial growth. This offers some microbiological safety by decreasing the growth of pathogenic organisms that may endure the thermal processing. Also, this helps assure adequate shelf life of the product by preventing the growth of spoilage microorganisms. However, heat and nutrients from the product often infuse the brine allowing bacteria such as *L. monocytogenes* and lactic acid bacteria to survive, grow and spread in these recycled chilling brines. Consequently, it is also critical to maintain the microbial safety of the cooling brines, especially when the brine is recycled. Bacterial contamination of these brines has been directly linked to the outbreaks of listeriosis from commercial ready-to-eat foods. The addition of one or several steps prior to packaging could help to minimize growth of *L. monocytogenes* in ready-to-eat food products.

Ultraviolet (UV) light has been utilized for the control of microbial contamination in some areas of the food industry because of its bacteriocidal effects. Ultraviolet radiation emitted between wavelengths of 220-300 nanometers is considered to have a germicidal effect on bacteria, molds, yeasts and viruses (65). The most microbiologically damaging wavelength range of ultraviolet radiation is between 240 and 280 nanometers.
Gram-negative bacteria are most easily killed by UV, while Gram-positive bacteria, spores and molds are much more resistant. The germicidal properties of UV radiation are mainly due to DNA mutations induced through absorption of UV light by the nucleic acids on the DNA strand of the microorganism. The cross-linking of thymine dimers on the DNA strand prevents repair and reproduction (35).

The Food and Drug Administration has approved UV treatments for pathogen reduction in water. The use of UV is a promising bactericidal alternative for other applications because it does not undesirably affect the color, flavor, odor, or taste of the product (92). Also, UV radiation does not produce undesirable by-products and is effective against a wide variety of microorganisms (27). The objective of this research was to use ultraviolet irradiation within a model brine chiller system to inactivate *L. monocytogenes* and lactic acid bacteria species. In using UV irradiation to reduce the bacterial load in recycled chilling brines, the safety of these ready-to-eat meat products will be improved due to the decreased risk of foodborne listeriosis. Also, the shelf life of the product will be increased by the reduction of spoilage lactic acid bacteria.
REVIEW OF LITERATURE

A. *Listeria monocytogenes*

1. Characteristics

*Listeria monocytogenes* is a well-known foodborne pathogen that is ubiquitous in nature. It is commonly found in water and soil, but has also been isolated from a number of domestic and wild animals. *Listeria monocytogenes* has been found in raw foods, such as uncooked meats, vegetables, fruits, and dairy products made from unpasteurized milk (25). More importantly, *L. monocytogenes* has been associated with processed foods, most commonly ready-to-eat (RTE) meat products, such as frankfurters or deli meats. *Listeria monocytogenes* is a short (0.5µm in diameter by 1 to 2µm long) Gram-positive, non-sporeforming rod that is a part of the lactobacilli family. It has tumbling end-over-end motility at room temperature (13). It is catalase positive, oxidase negative, and has slight β-hemolysis on blood agar. Although it has optimum growth at ~32°C, it can survive and multiply at refrigeration temperature. *Listeria monocytogenes* has also been known to survive freezing and drying, which provides difficulties for the food industry (25).

2. Illness

In the United States, approximately 2,500 individuals become seriously ill with listeriosis each year, and approximately 500 of those people die (21). Although *L. monocytogenes* is generally known to cause severe illness, there have been outbreaks in which the majority of the patients only developed mild symptoms, as the bacteria generally do not affect healthy adults or children (25). The infective dose of *L. monocytogenes* is unknown. However it is assumed that less than 1,000 total organisms could cause infection depending on susceptibility of the victim (36). *Listeria monocytogenes* is of major concern for several high-risk subpopulations, such as the elderly, the perinatal, and those who are immunocompromised (25). Approximately 12 hours after ingestion of the
contaminated food, those inflicted exhibit mild influenza-like symptoms including fever, headache, nausea, vomiting, and diarrhea (36). Healthy adults and children rarely become seriously ill. However, in the high-risk subpopulations, the mild symptoms are often followed a few days to a few weeks later by more serious complications. These can include meningitis, encephalitis, septicemia, and intrauterine/cervical infections that may result in stillbirth or miscarriage in pregnant women (25).

3. Foodborne Outbreaks

Pasteurization and heating methods used during the preparation of ready-to-eat food products will kill *L. monocytogenes*. However, post-process contamination occurs because the microorganism can readily adapt to and live in food processing environments. Most cases of listeriosis occur as sporadic incidents; however there have been many significant outbreaks of the illness. Most recently, in October of 2002 an outbreak involving eight Northeastern states brought about the largest meat recall in history. Pilgrim’s Pride Foods recalled 27.4 million pounds of cooked deli products produced in a Pennsylvania factory over a five-month period. This multistate outbreak of *L. monocytogenes* infections included 46 culture-confirmed cases, seven deaths and three stillbirths or miscarriages (23). In November 2000, homemade Mexican Style soft cheese was reported as the contaminated food source in a listeriosis outbreak involving 12 individuals in North Carolina. In this outbreak, ten of the cases involved pregnant women and resulted in five stillbirths, three premature deliveries, and two infected newborns (22). Also that same year, deli turkey and chicken meat was associated with 29 illnesses due to this organism in 10 states between May and November (21). In August of 1998, 40 illnesses caused by a single strain of *L. monocytogenes* were identified in ten states. This outbreak was linked to high environmental levels of *L. monocytogenes* in the production facility where the contaminated deli meats and frankfurters were processed (20). Mexican style soft cheese was responsible for at least 86 cases and 29 deaths due to foodborne listeriosis in Los Angeles and Orange counties in California in 1985 (19).
4. Factors Affecting Growth and Survival in ready-to-eat meat products

a. Temperature

There are many factors that will affect the growth and survival of *L. monocytogenes* in ready-to-eat meat products. *Listeria monocytogenes* can survive longer than many other non-spore forming bacteria under adverse environmental conditions (49). Temperature is one factor that may help support the growth of *L. monocytogenes* in meat products. *Listeria monocytogenes* is able to grow over a broad temperature range. Lower and upper limits for growth are approximately 1°C and 45°C, respectively. The optimum growth temperature for *L. monocytogenes* is 30–37°C (79). The ability of *L. monocytogenes* to grow at low temperatures allows for the possibility of proliferation in refrigerated food products. This is why it is necessary for strict temperature control to minimize *L. monocytogenes* growth. Glass and Doyle (40) found that *L. monocytogenes* could survive on several types of processed meat products that were stored at 4.4°C, including ham, bologna, wieners, sliced chicken and turkey, and sausages. The growth rate depended largely on the type of product and proliferation was greatest on processed poultry products. Another study found that 65.6% of wieners inoculated with *L. monocytogenes* supported growth of the pathogen while stored under vacuum at 5°C for up to 28 days (59).

Temperature will often affect the generation time of bacteria by slowing down replication. Papageorgiou et al. (70) reported generation times of *L. monocytogenes* in whey cheeses at 5°C ranged between 16.2 and 20.2 h and were significantly longer than those observed at 12°C, which ranged between 5.1 and 5.8 h. Generation times at 22°C ranged between 1.7 and 2.7 h. Andrews and Grodner (1) reported a similar generation time of 1.2 h in tryptic soy broth at 20°C.
Temperatures may fluctuate wildly during storage of a food product and this can lead to an increased risk of proliferation of pathogenic bacteria. A study by Bovill et al. (11) examined the growth of *L. monocytogenes* during various rates of increase and decrease in temperature. The group found that growth was not affected by even the most rapid changes and injury or lag was not observed. However, another study found that *L. monocytogenes* that was heat shocked in ground beef at 46°C for 60 min had elevated D_{10}-values as compared to nonheat-shocked controls. Refrigeration and frozen storage did not influence the observed effects (67).

In addition to the ability to grow at refrigeration temperature in many foods, *L. monocytogenes* can tolerate and grow in foods with high salt concentration, as well as in foods with low moisture content, and in comparatively acidic foods (49).

**b. Salt Concentration**

Sodium chloride is an important microbial inhibitor that has been reported as preventing the recovery of stressed *L. monocytogenes* on solid agar media at concentrations of 2% (58), 4% (60), 4.5% (28) and 6% (17). Hudson (46) examined the effectiveness of high sodium chloride concentrations for the destruction of *L. monocytogenes*. It was found that at relatively low NaCl concentrations (6%), *L. monocytogenes* would grow at refrigeration temperature. Numbers could be reduced in the presence of 26% NaCl, but not under practical time periods for use in the food industry. In another study, Peterson et al. (71) used sodium chloride and packaging methods to control *L. monocytogenes* in smoked fishery products. The group found that inhibition related to NaCl concentration was most apparent at 5°C and the *L. monocytogenes* populations were held below 10^2 CFU/g by 6% NaCl.
Many studies have been conducted to determine the growth and survival of *L. monocytogenes* under multiple constraints. In one such study, Buchanan et al. (16) studied the effects and interactions of several factors on the growth of *L. monocytogenes* including temperature, pH, atmosphere, sodium nitrite and salt concentration. It was found that although refrigeration was the primary factor in controlling the rate of growth, NaCl also had an effect on growth. The combined bacteriostatic effects that were most effective proved to be a combination of pH 6.0, 4.5% NaCl, 5°C incubation, and anaerobic conditions (cultures flushed with sterile N₂ for 10 minutes and sealed with screw caps). It has also been found that the combined effect of salt concentration (2 to 3.5%) and low temperature (10°C) seem to play a protective role, allowing *L. monocytogenes* to better survive exposure to nisin, a bacteriocin (33).

Bal’a and Marshall (3) found that *L. monocytogenes* tolerance to NaCl was greatest (>78 g/liter) at neutral pH (6.8 to 7.2) and increased in the pH range 7.0 to 5.4 as the incubation temperature was lowered to 5°C.

c. Water Activity

Another factor allowing for the growth and survival of *L. monocytogenes* is the ability of the organism to multiply at an unusually low water activity (aₜ). It is reported that the type of solute as well as the osmotic conditions created by the solute effect the ability of *L. monocytogenes* to grow. Tapia de Daza et al. (86) observed aₜ minima for strains Scott A and Brie 1. Both strains grew well at 30°C in glycerol-supplemented TSB, but not in NaCl- and sucrose-supplemented TSB at aₜ 0.90. The effects of all three solutes were magnified at 4°C. In a separate study, Petran and Zottolla (72) were able to grow *L. monocytogenes* in a 39.4% sucrose solution with a water activity of 0.92. A few years later these findings were duplicated by Farber et al. (34) reporting that the
minimum $a_w$ that permitted growth of the *L. monocytogenes* was 0.92 in a TSB base with sucrose as the humectant.

Miller (61) reported that survival of *L. monocytogenes* is related to the $a_w$ level, as well as the solute employed. He used brain heart infusion (BHI) broth adjusted to water activity levels of 0.99-0.80 with glycerol, NaCl, or propylene glycol and found minimum $a_w$ levels for growth occurred at 0.90, 0.92, and 0.97, respectively. The use of NaCl in this study was examined in greater detail then the other solutes because of its common use in foods. It was found that when using NaCl as the solute, growth of *L. monocytogenes* occurred at water activities of 0.99, 0.97, 0.93, and 0.92. It was also reported that below $a_w$ levels of 0.92 the death rate of *L. monocytogenes* Scott A was proportional to water activity. Survival of the organism in NaCl at 28°C was estimated to range from 200-700 hours, depending on the water activity. Nolan et al. (66) obtained similar $a_w$ minima using tryptic soy broth with 0.6% yeast extract (TSB-YE) containing glycerol and NaCl; in addition the group obtained an $a_w$ minimum of 0.92 using sucrose.

Chen and Shelef (29) used a model meat system to show the relationship of several factors including water activity on the growth of *L. monocytogenes*. The pair reported inhibition of strain Scott A in cooked strained beef having an $a_w$ of 0.93, a moisture content of 25%, and no added solutes. As mentioned earlier, *L. monocytogenes* is known for its ability to survive in many environments in which other pathogens cannot. As shown by the finding in these studies, it can be concluded that *L. monocytogenes* is a serious threat as a foodborne pathogen because is it able to grow at $a_w$ values <0.93 (49).

d. pH

In addition to salt concentration and water activity, pH also affects the growth and survival of *L. monocytogenes*. The organism has been
shown to grow over a wide pH range. The optimum pH range for *L. monocytogenes* growth is 6.0–8.0. However, growth of *L. monocytogenes* is possible in pH ranges from 4.1-9.6 (49). Parish and Higgins (69) examined the survival of several *L. monocytogenes* strains in pH adjusted TSB with yeast extract (TSB+YE). Growth was observed in the TSB+YE at pH 4.5 and higher for all strains during incubation at 30ºC and growth did not occur at pH 4.0 or lower. It has been found that the minimum pH supporting the growth of *L. monocytogenes* is dependent upon the temperature of incubation, nutrients available, moisture content, and the composition of the food product (49). Bal’a and Marshall (3) studied the combined effects of pH and salt on the survival of *L. monocytogenes* using double-gradient plates. The pH gradient across the plates ranged from 5.4 to 7.8 and the salt gradient ranged from 25 to 78 g/liter. The plates were incubated at 5, 15, 25, or 35ºC. The largest area of growth inhibition was observed on the salt-pH gradient plates incubated at 25ºC. At all incubation temperatures, peak salt tolerance occurred around neutral pH. It was found that *L. monocytogenes* was able to tolerate higher salt concentrations and a lower pH as the incubation temperature was lowered. The pH range for meat products ranges from 5.1–6.4, depending on the animal of origin and how the meat is processed (49). Since the pH range of meat products falls within the range of growth for *L. monocytogenes*, contamination of ready-to-eat meat products is a concern.

e. **Microbial Competition**

As a psychrotrophic pathogen, *L. monocytogenes* can often be isolated at low levels from a range of foods, but is rarely present at elevated numbers. The growth and survival of *L. monocytogenes* is also impacted by the growth of competing microorganisms (15). These organisms compete for available nutrients and consequently have an effect on product safety and stability through storage (55). The *Pseudomonas*
spp. are an example of psychrotrophic, Gram-negative spoilage organisms that are likely to be found in contaminated meat products stored aerobically. Buchanan and Bagi (15) found the suppression of *L. monocytogenes* by *P. fluorescens* was generally associated with low incubation temperatures (4°C) and sodium chloride levels (5 and 25 g/l) while slight increases of the maximum population density (<1.0 log cfu/ml) were observed when *L. monocytogenes* was grown in the presence of *P. fluorescens* at higher temperatures (12 and 19°C) and sodium chloride levels (25 and 45 g/l).

However, when the growth of these Gram-negative organisms is suppressed, there may be an increase in the proliferation of lactic acid bacteria such as the lactobacilli, pediococci, leuconostocs, and streptococci (12). These lactic acid bacteria also compete with *L. monocytogenes* in refrigerated, ready-to-eat meat products, often hindering listerial growth through the production of both lactic acid and bacteriocins. The antimicrobial activity of lactic acid is related to pH, and the undissociated form of the acid. In the undissociated form, the lactic acid can penetrate the cell membrane more easily. Once inside the cell, the acid dissociates because the cell interior has a higher pH than the exterior. Protons generated from intracellular dissociation of the lactic acid must be removed from the cell, using energy in the form of ATP. The constant entry of these protons will eventually deplete cellular energy and lead to cellular death (32). Bacteriocins are agents (commonly proteins) encoded in the genetic material carried on plasmids, produced for the purpose of inhibiting or killing closely related species, or even different strains of the same species (64).

In a separate study, Buchanan and Bagi (14) found that the growth of *L. monocytogenes* was suppressed when grown in the presence of two strains of *Carnobacterium piscicola*. Juven et al. (50) reported inhibition of *L. monocytogenes* in vacuum-packaged ground beef stored at 4°C using a lactic acid producing strain (FloraCarn L-2) of *Lactobacillus*.
alimentarius. Berry et al. (7) observed a 2 log10 CFU/g reduction of L. monocytogenes by a bacteriocin-producing Pediococcus in fermented semidry sausage.

B. Lactic Acid Bacteria

1. Characteristics

The lactic acid bacteria (LAB) consist of a number of genera including Carnobacterium, Enterococcus, Lactococcus, Lactobacillus, Lactosphaera, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragnococcus, Vagococcus, and Weissella (47). Carr et al. (18) defined the LAB as “Gram-positive, aerobic to facultatively anaerobic, asporogenous rods and cocci which are oxidase, catalase, and benzidine negative, lack cytochromes, do not reduce nitrates to nitrite, are gelatinase negative, and are unable to utilize lactate.” Although the lactic acid bacteria group is composed of a number of separate genera, they are classified as either homofermenters or heterofermenters. The differences between these two groups are the major end products after the bacteria ferment glucose. The homofermenters produce lactic acid as a major product of the fermentation, where the heterofermenters produce several other products besides lactic acid, including carbon dioxide, acetic acid, and ethanol (74).

The LAB are often used beneficially in food products. The LAB are hardy microorganisms and are often able to survive the stressors provided by the environment of the food product. They can be used as starter cultures in fermented foods or as preservatives by exploiting their ability to product bacteriocins (74). In a study sampling retail food products, Garver and Muriana (39) found by direct plating, bacteriocin-positive LAB were detected at levels up to 2.4 × 10^5 CFU/g in ready-to-eat meats. Of those identified the homofermentive Lactobacillus curvatus (four strains) and Lactococcus lactis (nine strains) were the only isolates inhibitory to foodborne pathogens including L. monocytogenes.

However, because LAB are able to grow anaerobically, at low temperatures, and high salt concentrations these organisms can also survive and
grow on food products and lead to spoilage and decreased shelf life. Some LAB have been found to be responsible for souring, slime formation, and off-color and off-flavor production in meat products (41). Samelis et al. (77) found that both the growth rate and the composition of spoilage LAB during refrigerated (4°C) storage of cooked, cured meats, sharing their processing plant environment, day of production and film packaging conditions were dependent on the product type and manufacturing method. Apart from the effect of pH, moisture, salt (brine) or sugar content, the cooking method strongly affected the LAB, as their growth was more delayed in smoked meats than in steam-cooked meats.

2. Shelf life and spoilage

The shelf life of meat and meat products is determined by the amount of time the product can be stored until spoilage occurs. The meat is considered spoiled when a maximum acceptable bacterial level is reached or an unacceptable off-odor or off-flavor and/or undesirable appearance occurs (10). However, even if high bacterial numbers are reached there may not be apparent spoilage of the product. For example, in a study by Susiluoto et al. (84) found that the average CFU/g of 32 packages of marinated broiler meat products at the end of the producer-defined shelf life was found to be 2.3x10⁸ on Plate Count Agar (PCA). Despite high bacterial counts, radical spoilage changes such as unpleasant odor, slime production and formation of gas were not seen.

The various forms of microbiological spoilage are preventable to a large degree by a wide range of preservation techniques, most of which act by preventing or inhibiting microbial growth, such as chilling, freezing, drying, curing, packaging, and adding preservatives (42). Since ready-to-eat meat products are heat processed to high temperatures, most vegetative cells are destroyed and it is post-process contamination that strongly influences the shelf life of the product (10).

Throughout storage, environmental aspects such as temperature, atmosphere, pH and salt content will select for specific microorganisms, and
influence their growth rate and activity. Thus allowing for a varied shelf life
for refrigerated meat and meat products, lasting from only a few days to
several months (10). The shelf life of some meat products is also determined
by intrinsic factors of the meat. Blixt et al. (9) found high correlations
between initial values of pH, fat and L-lactate, respectively, and the rate of
spoilage. However, no relationship was found between spoilage and the
origin of the meat (pork or beef).

3. **Carnobacterium gallinarum**

   Previously known as “atypical meat lactics” several lactic acid bacteria
have been installed in a new genus, *Carnobacterium*. This reclassification
was due to DNA homology and physiological studies and includes organisms
such as *Lactobacillus divergens*, *Lactobacillus piscicola*, and a few other rod-
shaped psychrophilic microorganisms (31). *Carnobacterium gallinarum* is a
Gram-positive, nonsporeforming, short, stubby rod often found in pairs or
short chains. It has no motility at 25°C, is catalase negative and shows no
hemolysis on blood agar (55). The organism has the ability to grow at 0°C,
but not at 45°C and is inhibited by acetate and by pH <6.0 with an optimal pH
in the range of 8.0 to 9.0 (18).

   The *Carnobacteria* have been largely associated with meat spoilage,
predominantly poultry. Researchers have also isolated these microorganisms
from chilled, vacuum-packaged, unprocessed beef, lamb, and pork (18). In a
study by Hansen and Huss (43) three *Carnobacterium spp.* produced spoilage
off-odors in spoiled cold-smoked salmon, resulting in 9% of the LAB isolates
being identified as *Carnobacterium spp.* This is in contrast to other
screenings of cold-smoked salmon in which the *Carnobacteria* played a much
more dominate role in the spoilage of those products. Paludan-Müller et al.
(68) found the LAB microflora was dominated by *Carnobacterium piscicola*,
which was found to account for 87% of the 255 LAB isolates from cold-
smoked salmon stored at 5°C. Similarly, Leroi et al. (56) found
Carnobacterium piscicola to be widely represented (97/155 LAB isolates) on cold-smoked salmon stored at 8°C.

In addition to causing spoilage problems in cold stored seafood, Carnobacterium spp. are often associated with the spoilage of refrigerated meat products. Barakat et al. (4) found the Carnobacteria to be one of the most prevailing isolates (71/203 LAB isolates) from cooked, modified atmosphere packaged, refrigerated, poultry meat.

4. Enterococcus faecalis

Bacteria in the genus Enterococcus have been recognized since the late 1800’s, when they were describes as the ‘entérocoque’ to give emphasis to their intestinal origin (81). Carr et al. (18) defined the enterococci as “Gram-positive, facultative anaerobic cocci with growth at 10°C and 45°C, growth in broth with 6.5% NaCl, growth at pH 9.6 and reduction of 0.1% Methylene Blue Milk Medium.” The Enterococci generate lactic acid homofermentatively from glucose and also gain energy from the break down of amino acids. As a normal inhabitant of the human intestinal tract, the Enterococci, usually Enterococcus faecalis, have been associated with urinary tract infections, bacteremia, bacterial endocarditis, and nosocomial infections (81).

There is an elevated likelihood for contamination of meat at the point of slaughter due to the existence of enterococci in the gastrointestinal tract of animals. Stiles et al. (80) completed a study of enterococci from raw meat products and found E. faecalis was the major isolate from both beef and pork cuts. The group also found another enterococci, E. faecium, was frequently isolated from bologna. In a study of three hog slaughtering plants, Knudtson and Hartman (52) found mean populations of $4 \log_{10}$ and $8 \log_{10}$ enterococci per 100 cm$^2$ of carcass surface at various stages in the slaughtering process, indicating that microbial contamination of meat products can occur at any point between slaughter and processing. Enterococcus faecalis (79%) was the most abundant Enterococcus spp isolated.
Enterococci are among the most thermotolerant of the non-spore-forming bacteria and this often leads to spoilage problems in cooked, processed meats. Processed meats are usually salted or cured and can be either raw or cooked (38). After surviving heat treatment, both E. faecalis and E. faecium have been implicated in spoilage of cured meat products (5, 6).

5. Lactobacillus plantarum

In the genus Lactobacillus, there is a classic division based on fermentative characteristics: (1) obligately homofermentative; (2) facultatively heterofermentative; and (3) obligately heterofermentative. Several species of groups 1 and 2 and some from group 3 are used in fermented foods. But the lactobacilli in group 3 are also commonly associated with food spoilage. Lactobacillus plantarum falls into group 2 because of its use as a starter culture for some fermented sausages and cereal products. However, it has been known to cause spoilage problems in some food products (81).

Lactobacillus plantarum is a Gram-positive, non-spore-forming short rod. It is often found singly or in pairs, but can also form small chains with short round ends (55). Lactobacillus plantarum is considered a psychrophilic microorganism because it can grow at temperatures of 2°C to 8°C, although they grow very slowly at these temperatures (18). This organism is a member of a large group of organisms known as the Streptobacteria. The Streptobacteria are found in nature connected with a variety of plants and dairy products. Lactobacillus plantarum has been associated with both types of products, including cabbage and cheese. Typical Streptobacteria, such as L. plantarum, ferment both lactose and maltose, but differ from Atypical Streptobacteria by also fermenting mannitol (18).

Although Lactobacillus spp. are naturally occurring in some food products, they are also an important cause of spoilage in the meat industry. In one study, Samelis et al. (78) determined that Lactobacillus sakei was the most prevalent species in the spoilage microflora of sliced, vacuum-packed, smoked, oven-cooked turkey breast fillets which developed slight, sour
spoilage flavors after 4 weeks storage at 4°C. In an experiment to determine the influence of packing atmosphere on the microbial spoilage pattern of ground beef, it was found that of the Gram-positive isolates, the lactobacilli predominated at 45% of isolates. The researchers found significant numbers of lactobacilli from samples in all treatment groups, including the aerobically packed categories (88).

The *Lactobacillus* spp. are fairly heat sensitive organisms. Franz and von Holy (37) found a one-log reduction in numbers on vienna sausages was achieved at 57°C in less then 60 seconds. In their experiment, the researchers determined D-values at 57, 60 and 63°C for *Lactobacillus sake* were 52.9, 39.3 and 32.5 seconds, respectively, and for *Lactobacillus curvatus* D-values were 22.5, 15.6, and 14.4 seconds, respectively, in vacuum packaged vienna sausages.

C. **Recycled Chill Brines**

1. **General**

   Thermally processed foods are often cooled using recycled brine in order to rapidly remove heat that could expose the product to temperatures that allow for bacterial growth. This cooling is done in order to increase shelf life by decreasing growth of spoilage microorganisms and it also aids in food safety by decreasing the growth of pathogenic organisms that may have contaminated the product. However, the heat and nutrients from the product often infuse the brine, leaving the brine as a potential habitat for these microorganisms. In addition to this, these brines are often recycled, which could lead to contamination of the thermally processed food while it is being cooled (62).

   USDA regulations state that brine may be reused to chill cooked product for various lengths of time based on salinity and temperature. The solution maintenance requirements for recycling the brines are shown below. The provisions range from recycling the brines for one production shift with
no temperature or sodium chloride controls, to reuse up to 4 weeks, requiring maintaining 20% NaCl and a maximum temperature of $-12.2^\circ$C (87).

**Table 1**: USDA regulations for recycled chill brines (USDA, 2000)

<table>
<thead>
<tr>
<th>Duration of use</th>
<th>Minimum salt Concentration (%)</th>
<th>Maximum temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>One production shift</td>
<td>None</td>
<td>Undefined</td>
</tr>
<tr>
<td>Up to 24 hours</td>
<td>5</td>
<td>40°F (4.4°C)</td>
</tr>
<tr>
<td>Up to 1 week</td>
<td>9</td>
<td>28°F (-2.2°C)</td>
</tr>
<tr>
<td>Up to 4 weeks</td>
<td>20</td>
<td>10°F (-12.2°C)</td>
</tr>
</tbody>
</table>

2. **Survival of *L. monocytogenes* in Recycled Chill Brines**

Contamination of *L. monocytogenes* in recycled chill brines is of great concern to the food industry. Many studies have been completed investigating the survival of *L. monocytogenes* in brines. Larson et al. (54) obtained commercial cheese brines from factories and inoculated them with *L. monocytogenes*. Survival of the pathogen ranged from <7 days to over 259 days and showed no proliferation. In addition to just evaluating the survival of *L. monocytogenes* in brines, several processes have been explored for their applications for the inactivation of the organism, such as microfiltration (44), organic acids (73), and electrochemical treatment (91). In one study, Miller et al. (62) used a model brine system to evaluate the growth, injury, and survival of *L. monocytogenes*. The organism grew at 5°C in 5% NaCl and at 12°C in 9% NaCl and there was no significant injury observed. Bacteriostatic conditions were maintained for the pathogen at -2°C and 9% NaCl. However, *L. monocytogenes* survived for 30 days at -12°C in 20% NaCl. In another study using a model brine system, Ye et al. (91) examined using electrochemical treatment system consisting of a pulsed electrical power supply and an electrical treatment chamber to inactivate *L. monocytogenes* in recirculated brine for chilling processed bacon. The team used both fresh
brines and used brines (after 20 hours of operation in the plant) at several different
temperatures. The average D-value was 1.61 min for the fresh brines at 7mA/cm\(^3\)
and 2.5 min at 35 mA/cm\(^3\) for the used brines.

D. Ultraviolet (UV) radiation

1. General

One physical method of sterilization of surfaces, equipment, and food
products is the use of ultraviolet energy. This is done by using radiation from the
ultraviolet region of the electromagnetic spectrum for the purpose of disinfection.
Although the UV spectrum includes wavelengths between 100 nm and 400 nm, it
can be subdivided into 4 sections: UVA, UVB, UVC, and the vacuum UV range.
The section of interest is the UVC (200 to 280 nm) due to its antimicrobial
activity. Within this UVC range, the wavelengths around 260 nm are the most
effective in inactivating bacteria and viruses, since at this wavelength DNA
mutations are induced through UVC absorption by the nucleic acids (26).

DNA absorption of the UVC light causes crosslinking between
neighboring pyrimidine bases on the same DNA strand, and thus formation of
hydrogen bonds with the purine bases on the other strand is impaired and DNA
transcription and translation is blocked, leading to cell death (63).

UV inactivation of microorganisms is mainly dependant on the UV
dosage and not the intensity of the light. The actual dosage in µW·s·cm\(^{-2}\) required
to attain a specific level of microbial inactivation can be expressed as the product
of applied intensity (µW/cm\(^2\)) and irradiation time (s). It is thus implied that for a
given UV dose at low radiation intensity and long exposure time, the same effect
is achieved with high intensity for a short time (65).

2. Susceptibility of Microorganisms

Generally, the UV dose required to inactivate viruses and molds is much
higher then for bacteria (27, 65). However, resistance of bacteria differs between
the species and also depends on age of the organisms and the presence of spores.
In general, Gram-positive bacteria tend to be more resistant to UV radiation than
Gram-negative organisms and spore formers are more resistant than non-spore formers. Vegetative bacteria tend to be most resistant to UV radiation just prior to active cell division, during the lag phase. It has also been found that a given dose will become less effective the higher the number of cells. The absence of oxygen will also increase microbial resistance to UV irradiation (48).

3. Efficiency and Application of UV Radiation

The use of UV for its germicidal properties in food is restricted due to the fact that UV acts only on the surface, except when the food product is transparent. Also, the only organisms to be killed will be those that are in the direct radiation beam. Food products are often not uniform in shape, which could cause shadows or holes and pores in which the UV light cannot penetrate (2).

UV rays are not capable of penetrating solid foods and have only a limited ability to penetrate liquids. There are several factors affecting the use of UV irradiation on liquids. The most critical factor is the transmissivity of the material being sterilized, as even small amounts of solutes or particulates will attenuate and scatter UV light, resulting in a lower measure of microbial inactivation. Also of importance is the thickness of the radiation path through the liquid because attenuation of the UV light is increased with the length of passage (26).

4. Applications

UV radiation has been used to disinfect drinking water and has shown effectiveness against a variety of microorganisms (2, 27). It has also been used to treat air and surfaces in hospitals and laboratories where aseptic facilities are required (30). UV radiation has also been used for the treatment of packaging materials for aseptic packaging (35).

A good deal of research has been completed on using direct application of UV light to food products. Kissinger and Willits (51) were able to reduce microorganisms in maple sap by 99% using UV energy. It has also been used effectively on shell eggs (53). Treatment with high intensity UV extended the shelf life of fresh mackerel fish by 7 days over the untreated fish (45).
Since UV light cannot penetrate into foods, only microbes on exposed surfaces are susceptible to its effects. Bacteria on a smooth surface absorb more UV light than bacteria on a rough surface (82, 89, 90). Consequently, the UV exposure necessary for destruction of bacteria on meat will most likely exceed that required for killing cells on laboratory media. Studies have shown that UV exposure does not have a deleterious effect on the color of meat nor does it cause oxidative rancidity (82, 89).

The use of ultraviolet radiation to control the growth of pathogens and spoilage microorganisms while avoiding unfavorable effects on the quality of the meat has been documented. Stermer et al. (82) reported that UV radiation dose of 150 µW·s·cm⁻² reduced bacteria on a smooth surface of fresh meat by 2 log cycles. In another study, Wong et al. (90) reported the greatest logarithmic reductions of bacteria on fresh pork muscle was achieved at doses of 100 µW·s·cm⁻² for *E. coli* and 80 µW·s·cm⁻² for *S. senftenberg* where a 1.5- and 2-log reduction was observed, respectively. Sumner et al. (83) were successful in almost completely eliminating bacteria on agar plates, with a 99.9% reduction of *S. typhimurium* at 2,000 µW·s·cm⁻². Reduction of bacteria was less successful on the surface of poultry skin with an 80.5% reduction of *S. typhimurium* obtained at 2,000 µW·s·cm⁻². Wallner-Pendleton et al. (89) found similar results on broiler chicken carcasses, with a 61% reduction in viable *S. typhimurium* observed in UV-treated chicken halves as compared with untreated halves. The UV dose in this study ranged from 82,560 to 86,400 µWs/cm².

5. Ultraviolet inactivation of *Listeria monocytogenes*

Very little research exists studying the inactivation of *L. monocytogenes* by ultraviolet energy. In one study, Yousef and Marth (92) exposed *L. monocytogenes* grown on tryptose agar to short-wave UV energy (100 µW/cm²) for a period of time ranging from 0.5 and 10 minutes. It was shown that the highest rate of inactivation occurred in the second minute and the rate of death diminished and essentially leveled off after 4 minutes. Inactivation of 90% of *L. monocytogenes* on the plate occurred with the average dose of 3.4 µW·s·cm⁻². In
another study, Bintsis et al. (8) exposed *L. innocua* (chosen to mimic the behavior of *L. monocytogenes*) to long-wave UV energy (~365 nm) in conjunction with photosensitizing compounds. It was found that in the presence of psoralen at a concentration of 5 mgl⁻¹, reduction of 99.8% of *L. innocua* was obtained over 60 seconds.

MacGregor et al. (57) studied the light inactivation of food-related pathogenic bacteria using a pulsed power source. The group reported that as few as 64 light pulses of 1 µs duration were required to reduce *L. monocytogenes* populations by 99%. Cell populations of *Listeria* were reduced by 7 log₁₀ orders at the upper exposure level of 512 µs. In a similar study, Rowan et al. (75) also examined the effects of high-intensity pulsed-light emissions on the survival of several bacterial types. The group used both high and low UV content to inactivate *Listeria monocytogenes* on the surface of tryptone soya-yeast extract agar. The results showed that with 200 light pulses of high-UV content, the microbial populations were reduced by 6 log₁₀ CFU/plate. The levels of resistance of the different bacteria varied, in which the Gram-positive bacteria were shown to be more resistant to the effects of UV than the Gram-negative bacteria.

### E. Conclusions

Recent research in food microbiology has focused on non-thermal processing alternatives for ready-to-eat (RTE) foods. Ultraviolet light treatment is widely recognized and proven method for pathogen reduction in water. The use of UV is a promising bactericidal alternative for some other fluid applications in the food industry because it does not undesirably affect color, flavor, odor or taste, depending on the product. UV disinfestations are efficient, effective, and economical.

Concentrated brine (salt/water) solutions are commonly used to chill RTE products. The brine solutions are recirculated and sprayed over warm product until a certain product temperature is attained, normally ~4°C. It is critical to maintain low bacterial levels in the brine systems for food safety and food preservation reasons.
In line treatment of brine with UV to provide the necessary level of disinfection would be a low cost alternative for decreasing brine bacterial counts. Validation of UV for brine systems would be beneficial for meat processing and provide a critical control point for HACCP plans to address pathogen reduction of post thermally processed product.
REFERENCES


87. USDA. Food Safety and Inspection Service. 1983. Reuse of water or brine cooling solutions on product following a heat treatment. MPI Bulletin 83-16. USDA, FSIS, Washington, DC.


Use of Ultraviolet Light for the Inactivation of *Listeria monocytogenes* and Lactic Acid Bacteria Species in Recycled Chill Brines

Karol M. Gailunas, Susan S. Sumner*, Christine Z. Alvarado, and Robert C. Williams

*Department of Food Science and Technology*
*Virginia Polytechnic Institute and State University*
*Blacksburg, VA*

KEYWORDS: Ultraviolet light, *Listeria monocytogenes*, lactic acid bacteria, brines

*Corresponding Author:* Mailing address: Department of Food Science and Technology, Virginia Tech, Blacksburg, VA 24061-0418. Phone 540-231-5280 Fax 540-231-9293. Electronic mail address: sumners@vt.edu
INTRODUCTION

The growth of *Listeria monocytogenes* on refrigerated, ready-to-eat food products causes a serious potential food safety hazard. Although the organism can be destroyed if heated to a high enough temperature, there may be contamination of the food product after it has been thermally processed (23). Ready-to-eat meat products that have received heat treatment followed by cooling in brine before packaging may supply a favorable environment for multiplication of *L. monocytogenes* because of the decrease in competitive microflora and the high salt tolerance of the organism (17). Due to the risk of cross-contamination, postprocessing treatments are needed to inactivate *L. monocytogenes* on ready-to-eat food products.

Recycled brine is frequently used in food processing plants to cool thermally processed products. The rapid cooling of the product minimizes the chance that the product will be exposed to temperatures that permit bacterial growth. This offers microbiological safety by averting the growth of pathogenic organisms that may endure the thermal processing. Also, this assures adequate shelf life of the product by preventing the growth of spoilage microorganisms. However, heat and nutrients from the product often infuse the brine allowing bacteria such as *L. monocytogenes* and lactic acid bacteria to survive, grow and spread in these recycled chilling brines. Consequently, it is critical to maintain the microbial safety of the cooling brines, especially when the brine is recycled. Bacterial contamination of these brines has been directly linked to the outbreaks of listeriosis from commercial ready-to-eat foods. The USDA’s Food Safety and Inspection Service (FSIS) issued MPI Bulletin 83-16, stating that brine may be reused to chill cooked product for various lengths of time based on salinity and temperature. They range from recycling the brines for one production shift to up to four weeks (19).

Ultraviolet (UV) light has been utilized for the control of microbial contamination in some areas of the food industry because of its bacteriocidal effects. The Food and Drug Administration has approved UV treatments for pathogen reduction in water. The use of UV is a promising bactericidal alternative for other applications because it does not undesirably affect the color, flavor, odor, or taste of the product (22). Also, UV radiation does not produce undesirable by-products and is effective against a wide variety
of microorganisms (5). The use of ultraviolet radiation to control the growth of pathogens and spoilage microorganisms while avoiding unfavorable effects on the quality of the meat has been documented. Stermer et al. (15) reported that UV radiation dose of 150 µW·s·cm⁻² reduced bacteria on a smooth surface of fresh meat by 2 log cycles. In another study, Wong et al. (20) reported the greatest logarithmic reductions of bacteria on fresh pork muscle was achieved at doses of 100 µW·s·cm⁻² for E. coli and 80 µW·s·cm⁻² for S. senftenberg where a 1.5- and 2-log reduction was observed, respectively. Sumner et al. (16) were successful in almost completely eliminating bacteria on agar plates, with a 99.9% reduction of S. typhimurium at 2,000 µW·s·cm⁻². Reduction of bacteria was less successful on the surface of poultry skin with an 80.5% reduction of S. typhimurium was obtained at 2,000 µW·s·cm⁻². During more recent times, high intensity UV-C lamps have become available and have enhanced the potential of destroying bacteria on foods.

The objective of this research was to use ultraviolet irradiation within a model brine chiller system to inactivate L. monocytogenes and lactic acid bacteria species. In using UV irradiation to reduce the bacterial load in recycled chilling brines, the safety of these ready-to-eat meat products will be improved due to the decreased risk of foodborne listeriosis. Also, the shelf life of the product will be increased by the reduction of spoilage lactic acid bacteria.
MATERIALS AND METHODS

A. Brine Preparation

The brine solutions were prepared in the laboratory facilities 24 hours before treatment with ultraviolet light. All equipment and containers used for brine manufacturing were cleaned using hot water and HC-10 Chlorinated Kleer-Mor® (EcoLab, St. Paul, MN) high foaming caustic cleaner and sanitized with hot water and Ster-Bac Quaternary Ammonium Sanitizer (EcoLab, St. Paul, MN) at 200 ppm prior to use. The brines were prepared by dissolving Cargill Top-Flo Evaporated Salt (99.8% purity) (Cargill Inc., Minneapolis, MN) into domestic water until a 7.9%w/w or a 13.2%w/w solution was attained.

Top-Flo Evaporated Salt is of food grade quality, complying fully with the standards for sodium chloride as set forth in the Food Chemicals Codex. The U.S. Department of Agriculture Food Safety and Inspection Service approved this salt for direct use in meat and poultry products. The salt contains water-soluble yellow prussiate of soda, which is added to improve caking resistance in accord with 21CFR 172.490. A chemical analysis of the salt can be found in Appendix I.

The 7.9%w/w brine was prepared by dissolving 4.74 kg of salt into 60 kg of tap water in a 30 gallon Nalgene® bucket. The 13.2%w/w brine was prepared by dissolving 7.92 kg of salt into 60 kg of tap water. The salt and water was hand-mixed until dissolved and placed in a 4°C cooler for 24 hours to yield a final brine temperature of ~10°C.

B. Inoculum preparation

1. Listeria monocytogenes

Six strains of Listeria monocytogenes (LM) were used in this study. Listeria monocytogenes Scott A, V7, LCDC, D43, Brie, and ATCC 19115 were obtained from the Department of Food Science and Technology (FST), Virginia Polytechnic Institute and State University (VPI&SU), Blacksburg, Virginia. The cultures were prepared by inoculating 100 ml of tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) supplemented with 0.6% yeast extract (YE; Difco)
(TSBYE) with one strain of *L. monocytogenes*, and incubating at 32°C for 48 hours. This procedure was done for each of the six strains being used. One loop of this culture was streaked onto Modified Oxford (MOX) agar (Oxford Medium Base plus Modified Antimicrobial Supplement; Difco) to ensure isolation and incubated at 32°C for 48 hours. A confirmed colony of *L. monocytogenes* from the MOX plate was transferred back into 100 ml of sterile TSBYE and incubated at 32°C for 48 hours and then streaked onto slants of tryptic soy agar (TSA; Difco) supplemented with 0.6% yeast extract (TSAYE). The *L. monocytogenes* stock cultures were maintained on slants of (TSAYE). All culture slants were stored at 4°C. Before use, one loopful of culture from the slants was grown in 10 ml TSBYE and was incubated at 32°C for 24 hours. Each of these strains was centrifuged at 10,000 x G for 5 minutes and the pellet was washed with 10 ml of either 7.9%w/w or 13.2%w/w sterile brine (Top-Flo Evaporated Salt, Cargill Inc., Minneapolis, MN and tap water). The washed cells were also centrifuged at 10,000 x G for 5 minutes and the pellet was suspended in 10 ml sterile brine. A cocktail of *L. monocytogenes* was prepared by combining equal proportions of each of the six strains suspended in brine into a sterile container and stored at 4°C for 24 hours.

2. Lactic Acid Bacteria

Three strains of lactic acid-producing bacteria (*Carnobacterium
gallinarum* (ATCC 49517) isolated from ice slush from chicken carcasses, *Lactobacillus plantarum* (ATCC 49445) isolated from ground pork, and *Enterococcus faecalis* (ATCC 29212; a human isolate) were used in this study. All were obtained from the American Type Culture Collection (Manassas, VA). *Carnobacterium gallinarum* and *L. plantarum* were chosen to represent typical post-processing contaminants, while *E. faecalis* was chosen to represent potential thermoduric nonsporeformers that may survive thermal processing. *Carnobacterium gallinarum* and *E. faecalis* stock cultures were maintained on slants of All-Purpose Tween agar (APT; Difco). *Lactobacillus plantarum* stock cultures were maintained on slants of deMan, Rogosa, and Sharpe agar (MRS;
Remel Inc., Lenexa, KS). All culture slants were stored at 4°C. Before use, one loopful of culture from each slant was grown in 10 ml APT broth (for *C. gallinarum* and *E. faecalis*) or MRS broth (for *L. plantarum*) and was incubated at 30°C for 24 hours. Each of these strains was centrifuged at 10,000 x G for 5 minutes and the pellet was washed with 10 ml of sterile brine (Top-Flo Evaporated Salt, Cargill Inc., and tap water). The washed cells were also centrifuged at 10,000 x G for 5 minutes and the pellet was suspended in 10 ml sterile brine. A cocktail of Lactic Acid Bacteria (LAB) was prepared by combining two equal proportions of each of the three strains suspended in brine into a sterile container and stored at 4°C for 24 hours.

C. Survival Studies

In order to determine how *L. monocytogenes* would respond to environmental stressors such as temperature and brine concentration, several growth studies were performed. In duplicate sterile bottles, 100 ml of tap water, 7.9%w/w brine, or 13.2%w brine were inoculated with ~5 log<sub>10</sub> CFU/ml of the six strain *L. monocytogenes* cocktail and incubated at 4°C, 25°C, or 32°C. The brines were then plated in duplicate on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE) at 0, 4, 8, 24, 48, 72, 96, 120, and 144 hours. The TSAYE plates were incubated at 32°C for 48 hours. After incubation, the colonies were counted and recorded as CFU/ml brine.

D. Ultraviolet Treatment System

The Ultraviolet treatment system used was an Ultra-Violet Water Treatment Unit (Aquionics Inc., Erlanger, KY) model number AMD 150B/1/2T D. The treatment was achieved by passing the brine through a stainless steel chamber containing one UV emitting arc-tube. The arc-tube is mounted in a quartz sleeve and fitted within the chamber allowing the brine to pass the sleeve on all sides. After treatment, the brine was returned to a reservoir and was continually pumped through the treatment system. A diagram of the UV treatment system is available in Appendix II. The
entire system was kept in a 4°C laboratory in order to allow the brine to remain chilled.

After the brine had been chilled for 24 hours, it was placed into the reservoir of the UV treatment system. The pump was started and the uninoculated brine was allowed to circulate within the system with exposure to UV light for 10 minutes in order to decompose free chlorine in the brines. The light was then turned off and the prepared cocktail was added to the brine, giving an approximate inoculum of $1.0 \times 10^6$ CFU/ml (6.0 log CFU/ml). As a control, the inoculated brine was allowed to circulate through the system with no exposure to ultraviolet light for 1 hour. Duplicate 50 ml samples were taken at 0, 1, 5, 15, 30, and 60 minutes. After 1 hour treatment, this brine was emptied from the UV treatment system and discarded. A new 60 liters of uninoculated brine was added to the reservoir. Prior to addition of the inoculum, uninoculated brine was allowed to circulate within the system with exposure to UV light for 10 minutes in order to decompose free chlorine in the brines. The brine was then inoculated as previously described with the inoculum. The inoculated brine was recirculated and pumped through the treatment chamber to be exposed to ultraviolet light at a flow rate of ~47 liters per minute. Samples were taken in duplicate at 0, 1, 5, 15, 30, and 60 minutes.

In the UV treatment system the brine temperature was taken at each sampling to monitor any changes. All samples were obtained by aseptically collecting 50ml samples of the brine out of the brine reservoir and placed into sterile 4 oz. Whirl-Pak® bags (VWR, Bridgeport, NJ).

E. Microbial Analysis

The experiment was performed as stated above with the 7.9% brine and LM cocktail, the 13.2% brine and LM cocktail, the 7.9% brine and the LAB cocktail, and the 13.2% brine and the LAB cocktail. Each brine and cocktail combination was analyzed in triplicate. The UV Treatment system was cleaned and sanitized between each repetition. The system was cleaned with fresh hot water (~15 gallons) and low foaming caustic cleaner, Klenzade FastPac 110: Mechanical and CIP cleaner (EcoLab, St. Paul, MN), at approximately 2% w/w according to manufacturers.
directions. It was then sanitized with fresh hot water and Ster-Bac Quaternary Ammonium Sanitizer (EcoLab, St. Paul, MN) at 200 ppm. Cleaning and sanitizing procedures are found in Appendix III.

1. *Listeria monocytogenes*

At the appropriate time during recirculation (0, 1, 5, 15, 30, and 60 min), two 50 ml samples were collected. There was a total of 12 samples taken during each repetition, two 50 ml samples at each time interval. From each 50 ml sample, an aliquot was removed and serially diluted with 0.1% peptone (Difco) water and pour plated in duplicate on Modified Oxford (MOX) agar (Oxford Medium Base plus Modified Antimicrobial Supplement; Difco) plates in order to enumerate only the *L. monocytogenes* (LM) present in the brine. The MOX plates were incubated at 32°C for 48. After incubation, the colonies were counted and recorded as CFU/ml brine in a database for later comparison.

Using an aerobic plate count (APC) method, the remainder of the 50 ml samples were analyzed to determine if any significant injury was caused to the *L. monocytogenes* while in the UV treatment system. Another aliquot was removed from the original 12 samples and was serially diluted with 0.1% peptone (Difco) water and pour plated onto duplicate trypticase soy agar (TSA; Difco) supplemented with 0.6% yeast extract (YE; Difco) (TSAYE) in order to enumerate any microorganisms present in the brine. The TSAYE plates incubated at 32°C for 48 hours. After incubation, the colonies were counted and recorded as CFU/ml brine in a database for later comparison.

2. Lactic Acid Bacteria

At the appropriate time during recirculation (0, 1, 5, 15, 30, and 60 min), two 50 ml samples were collected. There was a total of 12 samples taken during each repetition, two 50 ml samples at each time interval. From each 50 ml sample, an aliquot was removed and serially diluted with 0.1% peptone (Difco) water and pour plated in onto duplicate All-Purpose Tween (APT) Agar (Difco) containing 0.0032% (w/v) bromcresol purple (BCP) dye (Fisher Scientific, Pittsburgh, PA) (APT+BCP) plates in order to enumerate only the Lactic Acid
Bacteria (LAB) present in the brine. The APT+BCP plates were then incubated at 30°C for 48 hours to allow for sufficient growth of any organisms present. After incubation, the colonies were counted and recorded as CFU/ml brine in a database for later comparison.

F. Statistical analysis

At the end of the study, the effect of ultraviolet light exposure on the inactivation of *L. monocytogenes* and *lactic acid bacteria species* at different brine concentrations (7.9%w/w and 13.2%w/w) and its effect on microbial population (log CFU/ml) was statistically analyzed. The data gathered was compared to the control (no exposure to UV) to establish any significant change in inhibitory performance that may have occurred in the brines with regards to each cocktail. Reported values are averages from three independent trials converted to logarithmic units. Each replicate was preformed on different days. The different brine and cocktail combinations were analyzed separately by the general linear model procedure using SAS statistical software (SAS Institute Inc., Cary, N.C.). Tukey’s HSD was performed to determine significant differences ( = 0.05) between the mean (log CFU/ml) of the control brines and the brines exposed to UV light.
RESULTS AND DISCUSSION

Ultraviolet light (UV) was effective at reducing microbial populations of both *L. monocytogenes* and lactic acid bacteria (LAB) in recycled chill brines. *Listeria monocytogenes* inoculum concentrations averaged 6.10 log$_{10}$ CFU/ml for studies in 7.9%w/w brine and 6.01 log$_{10}$ CFU/ml for studies in 13.2%w/w brines. As seen in Tables 1 and 2, mean *L. monocytogenes* populations were significantly reduced after five minutes exposure to UV light in the model brine chiller as compared to the control, which received no UV light exposure (P<0.05). There was no significant statistical difference in log reductions of *L. monocytogenes* plated on the two different media MOX and TSAYE (P<0.05). Lactic acid bacteria inoculum concentrations averaged 5.94 log$_{10}$ CFU/ml for studies in 7.9%w/w brines and 5.81 log$_{10}$ CFU/ml for studies in 13.2%w/w brines. The lactic acid bacteria inactivation followed a similar trend as the *L. monocytogenes*. As seen in Table 3 mean lactic acid bacteria populations were also significantly reduced after five minutes exposure to UV light in the model brine chiller as compared to the control (P<0.05). There was at least a 4.5 log CFU/ml reduction in microbial populations seen in all treated brines after exposure to ultraviolet light for 60 minutes.

The graphs shown in Figures 1, 2, and 3 all depict similar inactivation curves for *L. monocytogenes* and lactic acid bacteria in brines as compared to their controls over a 60 minute period. The average microbial populations for controls remained relatively constant during the entire 60 minutes in the model brine chiller. The maximum rate of inactivation for treated brines occurred between one and fifteen minutes of ultraviolet exposure.

Survival studies were performed to determine how *L. monocytogenes* would respond to environmental stressors such as temperature and salt concentration. The brines and tap water were inoculated at ~5 log$_{10}$ CFU/ml. Figure 4 displays the pathogens response to salt concentration at refrigeration temperatures (4°C) over a six-day period. Refrigeration allowed for better survival of *L. monocytogenes* in the brines (7.9%w/w and 13.2%w/w) than in the tap water. This may be due to homeoviscous adaptation, which enables the cells to maintain membrane fluidity at a decreased temperature. As the
temperature decreases, the cell begins to synthesize and increased amount of mono- and diunsaturated fatty acids. This increase in the degree of unsaturation leads to a decrease in lipid melting point, suggesting this adaptation occurs to maintain the lipids in a liquid and mobile state, thereby allowing membrane proteins to continue to function. As long as the membrane proteins are functioning, the cell can tolerate the higher salt concentrations by intracellular accumulation of compatible solutes (7). Compatible solutes stabilize intracellular enzymes and other proteins and enable them to continue functioning when the water activity decreases. The solutes Listeria monocytogenes accumulates are glycinebetaine and carnitine (J2). Also, the membrane’s physical state can influence and/or control expression of genes, particularly those that respond to temperature. The production of cold shock proteins (CSPs) also contributes to an organism’s ability to grow at low temperatures. Listeria monocytogenes that has been cold-shocked from 37°C to 5°C have been found to produce 12 CSPs (3). As shown in Figure 5, both the brines and the tap water showed similar inactivation rates of the pathogen when stored at 25°C. At 25°C, all three concentrations showed complete inactivation of L. monocytogenes after 48 hours. This is consistent with results reported by Bal’a and Marshall (2) who found that the largest inhibition of L. monocytogenes occurred on salt-pH gradient plates incubated at 25°C and the smallest area of growth inhibition occurred on plates incubated at 5°C. As the temperature increased to 32°C (Figure 6), the L. monocytogenes survived the longest in the tap water, followed by the 7.9%w/w brine and survival was the shortest in the 13.2%w/w brine. This may be explained by the lower water activity in the 13.2%w/w brines and/or the expression of salt stress proteins by L. monocytogenes. Miller reported that growth of L. monocytogenes Scott A occurred at water activities of 0.99, 0.97, 0.93, and 0.92. He also reported that below a_w levels of 0.92 the death rate of L. monocytogenes Scott A was proportional to water activity (9). The water activity (a_w) of the brines was measured during each replication. It was found that the average a_w for the 7.9%w/w brines was 0.94 and the average a_w for the 13.2% w/w brines was 0.89. Duché and colleagues reported that when under osmotic stress at 3.5%w/v NaCl, L. monocytogenes produces 12 salt stress-induced proteins. The identified proteins belong in two groups: the salt shock proteins (Ssp), which are rapidly but briefly overexpressed, and the stress acclimation proteins (Sap), which are also rapidly induced but still overexpressed several
hours later (6). It can be concluded that at colder temperatures *L. monocytogenes* is more adaptable to environmental stressors, such as salt concentration. This adaptability is why *L. monocytogenes* contamination of recycled chill brines is important. Because of this adaptability, it is important that recycled brines receive some form of sterilization in order to avoid contamination of the product.

Other researchers have indicated that UV-energy inactivates bacteria exponentially and thus the typical death curve for microorganisms treated with UV is often described as sigmoidal (1, 13, 22). In this study, the use of UV to reduce microbial populations in brines led to the formation of a sigmoidal curve. The tail of inactivation curves has been explained in several different ways. The first explanation is the multiple hit phenomena described by Yousef and Marth (22), which states that the sigmoidal survival curve was accounted for on the basis of multiple UV hits on a single cell or single UV hits on multiple cells. The tailing has also been explained by the lack of homogeneous population (4). This occurs when the bacteria are not uniform in structure or composition throughout the cocktail due to the fact they are composed of several different strains. These strains all have distinctive characteristics that make them different and these differences may cause one strain to be more resistant to ultraviolet energy than another. Finally, the tail on the sigmoidal shaped inactivation curve may also be caused by the presence of suspended solids that may block the UV irradiation (13). Some such solids include food particles from the product being chilled with the brines and other environmental contaminants such as dust or dirt.

The tailing effect often seen in other death curves was not as prominent in this study. This may be due to the fact that other UV inactivation studies were performed on flat surfaces, where the bacteria would have direct contact with the UV energy. As shown in Figures 1, 2, and 3 microbial numbers in the brines do level slightly after 15 minutes of UV treatment, but the tail is not as pronounced as in other studies. This may be due to turbidity in the brines caused by air bubbles during recirculation, causing the UV light to be scattered and have less contact with the microbial cells leading to a slower rate of death. Perhaps if the length of time the brine was exposed to ultraviolet light was increased, this tailing effect would have been more prominent.
The brines inoculated with *L. monocytogenes* were plated on two types of media, MOX and TSAYE. This was done to determine the amount of injury that could be caused to the cells during UV treatment. The early exposure of bacteria to UV is believed to injure cells. As the dose of UV is increased, mutations occur in the bacterial DNA that obstructs cellular replication. When the DNA transcription and replication is blocked, the cellular functions are compromised and this eventually leads to death (13). Injured cells would be harder to grow on the MOX plates due to its selective nature. Therefore if a high number of cells were being injured instead of killed in the treatment system; the counts would be higher on the TSAYE plates. Statistical analysis of the microbial counts on both types of media indicated no difference; therefore there was no cellular injury.

As seen in Figures 2 and 3, both 7.9%w/w brine and 13.2%w/w brine show similar logarithmic reductions in *L. monocytogenes* when treated for the same amount of time in the UV treatment system. Figure 4 depicts similar log reductions of both brine concentrations inoculated with lactic acid bacteria. From the data presented, there is no statistical difference exhibited in the log reduction between 7.9%w/w brine and 13.2%w/w brine (P<0.05) with both types of microorganisms. However, the lactic acid bacteria seemed to be slightly more susceptible to salt concentrations than the *L. monocytogenes*. As shown on Table 3, in the control brines that had no exposure to UV, the mean lactic acid bacteria populations decreased by twice as much in the 13.2% brine after 60 minutes than in the 7.9% brines. This is consistent with previous studies, in which low levels of NaCl stimulated lactic acid bacteria growth but higher levels decreased lactic acid bacteria growth drastically (11, 14, 18).

In the UV treatment system the brine temperature was taken at each sampling to monitor any changes. The control brines averaged 11.3°C at the beginning of the one-hour treatment. During the hour of treatment the brine temperature increased an average of 4.0°C. The UV treated brines averaged 10.3°C at the beginning of the one hour treatment. During the one-hour treatment with UV light, the brine temperature increased an average of 15.5°C.

The pH of the brines was also monitored during each repetition of the study. It was found that all brines averaged a pH of 7.2. Sodium chloride is a neutral chemical
salt, and exhibits neither alkalinity nor acidity. Sodium chloride brines are highly ionized, and possess no buffering action. Because of this, the pH of sodium chloride brines depends, not on the concentration of the salt in the brine, but on the presence of minute quantities of foreign alkaline or acidic materials in the brine. These usually appear in the form of dissolved gases in the dissolving water. For example, the presence of CO₂ in the dissolving water can lower the pH of the salt brine from the neutral point of 7.0 to a value of 4.0–6.0. Similarly, ammonia in the water can raise the pH of the brine to the range of 8.0–10.0. The brines in this study were all made with tap water in the laboratory facility. Depending on where the brines in a processing facility are made, as well as how the product is processed, the pH of brines in industry usually ranges from 6.0 to 8.0.

Other researchers have obtained comparable results to this study using other methods of disinfecting recycled chiller brines. Ye et al. (21) used an electrochemical treatment system to treat brines over a 60 minute time period. The group found and average D-value of 2.5 min at all three temperatures (4, 0, and -8°C). Hart et al. (8) used microfiltration and were able to see ~3 log₁₀ CFU/ml reduction in microorganisms after 80 minutes of treatment.
CONCLUSIONS

Overall results indicate that inline treatment of chill brines with ultraviolet light (UVC) shows promise as a processing intervention to inactivate *L. monocytogenes* and lactic acid bacteria. This design of the lab-scale ultraviolet treatment system allows for a continuous inline treatment of the brine and *L. monocytogenes* and lactic acid bacteria may be effectively controlled without interrupting the chilling operation. When compared with an offline alternative, this form of treatment system could be easily implemented, and would not require extra brine, storage tanks, or pumping equipment. Validation for the use of ultraviolet light in processing systems can provide documentation for a critical control point within Hazard Analysis and Critical Control Point (HACCP) plans to address pathogen reduction of post thermally processed products.
TABLE 1. *Bacterial populations of L. monocytogenes (LM) plated on Modified Oxford Agar (MOX)*

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>7.9% w/w brine</th>
<th>13.2% w/w brine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>UV</td>
</tr>
<tr>
<td>0</td>
<td>6.10±0.07a*</td>
<td>6.05±0.01a</td>
</tr>
<tr>
<td>1</td>
<td>6.09±0.04a</td>
<td>5.65±0.07a</td>
</tr>
<tr>
<td>5</td>
<td>6.06±0.06a</td>
<td>4.11±0.57b</td>
</tr>
<tr>
<td>15</td>
<td>6.04±0.06a</td>
<td>2.37±0.37c</td>
</tr>
<tr>
<td>30</td>
<td>6.02±0.03a</td>
<td>2.16±0.53cd</td>
</tr>
<tr>
<td>60</td>
<td>6.00±0.03a</td>
<td>1.36±0.63d</td>
</tr>
</tbody>
</table>

*Within each brine concentration, mean values followed by different letters are significantly different (P<0.05)*
**TABLE 2.** Bacterial populations of *L. monocytogenes* (LM) plated on Tryptic Soy Agar supplemented with 0.6% Yeast Extract (TSAYE)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>7.9% w/w brine</th>
<th>13.2% w/w brine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (log CFU/ml ± SD; n=6)</td>
<td>UV (log CFU/ml ± SD; n=6)</td>
</tr>
<tr>
<td>0</td>
<td>6.14±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.12±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>6.14±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.79±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>6.09±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.20±0.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>6.08±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.41±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>6.08±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20±0.49&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>6.04±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.51±0.49&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Within each brine concentration, mean values followed by different letters are significantly different (P<0.05)*
**TABLE 3.** Bacterial populations of lactic acid bacteria (LAB) plated on All Purpose Tween Agar supplemented with 0.0032% w/v brom cresol purple dye (APT+BCP)

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>7.9% w/w brine</th>
<th>13.2%w/w brine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>UV</td>
</tr>
<tr>
<td>0</td>
<td>6.04±0.25<em>a</em></td>
<td>5.84±0.27*a</td>
</tr>
<tr>
<td>1</td>
<td>6.05±0.23*a</td>
<td>5.64±0.17*a</td>
</tr>
<tr>
<td>5</td>
<td>6.05±0.21*a</td>
<td>3.90±0.53*b</td>
</tr>
<tr>
<td>15</td>
<td>6.00±0.18*a</td>
<td>2.80±0.65*c</td>
</tr>
<tr>
<td>30</td>
<td>5.98±0.18*a</td>
<td>2.10±0.31*cd</td>
</tr>
<tr>
<td>60</td>
<td>5.89±0.28*a</td>
<td>1.49±0.10*d</td>
</tr>
</tbody>
</table>

* Within each brine concentration, mean values followed by different letters are significantly different (P<0.05)
Figure 1. Mean microbial populations of brine inoculated with a six-strain mixture of *Listeria monocytogenes* plated on Modified Oxford Agar (MOX) during 1 hour of UV exposure. Solid lines represent 7.9% w/w brine solutions; dashed lines represent 13.2% w/w brine solutions. 7.9% brine – no UV exposure (▲); 7.9% brine – with UV exposure (■); 13.2% brine – no UV exposure (●); 13.2% brine – with UV exposure (♦).
Figure 2. Mean microbial populations of brine inoculated with a six-strain mixture of *Listeria monocytogenes* plated on Tryptic Soy Agar (TSA) supplemented with 0.6% Yeast Extract (YE) during 1 hour of UV exposure. Solid lines represent 7.9% w/w brine solutions; dashed lines represent 13.2% w/w brine solutions. 7.9% brine – no UV exposure (▲); 7.9% brine – with UV exposure (■); 13.2% brine – no UV exposure (●); 13.2% brine – with UV exposure (♦).
Figure 3. Mean microbial populations of brine inoculated with a three-strain mixture of lactic acid bacteria plated on All Purpose Tween (APT) Agar supplemented with 0.0032% (w/v) bromcresol purple dye (BCP) during 1 hour of UV exposure. Solid lines represent 7.9% w/w brine solutions; dashed lines represent 13.2% w/w brine solutions.

7.9% brine – no UV exposure (▲); 7.9% brine – with UV exposure (■);
13.2% brine – no UV exposure (●); 13.2% brine – with UV exposure (♦).
Figure 4. Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 4°C over a 6-day period.
Figure 5. Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 25°C over a 6-day period.
Figure 6. Mean microbial populations of brine and tap water inoculated with a six-strain mixture of *Listeria monocytogenes* stored at 32°C over a 6-day period.
REFERENCES


AREAS FOR FURTHER RESEARCH

In summary, brines contaminated with *L. monocytogenes* and lactic acid bacteria may be disinfected using ultraviolet energy. One further area for investigation may include exposing the brines to ultraviolet light for periods of time longer than 60 minutes. In this study, there was never a decrease in microbial numbers below detectable levels and it is essential to know how much exposure to UV light is necessary for all microorganisms in the brines to be inactivated. Another area for further study may consist of obtaining used brines from a processing facility producing ready-to-eat meat products and subjecting those brines to an investigation similar to this one. The used brines are likely to contain a number of dissolved and suspended solids due to the particles from product permeating into the brine during processing. These solids are likely to scatter ultraviolet light rays and slow down the inactivation rate. Finally, a study in which *L. monocytogenes* and lactic acid bacteria are both inoculated into the same brine should be tested. The lactic acid bacteria may aid in the inactivation of *L. monocytogenes* in the brine through the production of bacteriocins.
APPENDIX I

Chemical Analysis of Top-Flo Evaporated Salt (99.8% purity). Supplied by Cargill Inc., Minneapolis, MN:

<table>
<thead>
<tr>
<th>Component</th>
<th>Units</th>
<th>Typical</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Chloride (dry)</td>
<td>%</td>
<td>99.86</td>
<td>99.80 min.</td>
</tr>
<tr>
<td>Calcium &amp; Magnesium (as Ca)</td>
<td>%</td>
<td>0.04</td>
<td>-</td>
</tr>
<tr>
<td>Sulfate (as SO₄)</td>
<td>%</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>Surface moisture</td>
<td>%</td>
<td>0.03</td>
<td>0.1 max</td>
</tr>
<tr>
<td>Copper (as Cu)</td>
<td>ppm</td>
<td>0.01</td>
<td>0.5 max</td>
</tr>
<tr>
<td>Iron (as free Fe)</td>
<td>ppm</td>
<td>0.2</td>
<td>2.0 max</td>
</tr>
<tr>
<td>Heavy Metals (as Pb)</td>
<td>ppm</td>
<td>&lt;1.0</td>
<td>2.0 max</td>
</tr>
<tr>
<td>Water Insolubles</td>
<td>ppm</td>
<td>165</td>
<td>200 max</td>
</tr>
<tr>
<td>Yellow Prussiate of Soda</td>
<td>ppm</td>
<td>5</td>
<td>13 max.</td>
</tr>
</tbody>
</table>

¹By difference of impurities
²110°C for 2 hours
³Anticaking agent (sodium ferrocyanide decahydrate)
APPENDIX II

Diagram of the Ultraviolet Treatment System:
APPENDIX III

Methods for cleaning the UV Treatment System:

Prior to cleaning, the UV lamp system was allowed to de-energize and cool down for at least 30 minutes. For safety, wearing chemical resistant rain suit, boots, gloves and eye/face protection is advised. The test solution (inoculated brines) was drained and the reservoir was rinsed with hot fresh water (~15 gallons). The fresh water was circulated for 2 minutes and drained. The reservoir was then filled again with fresh hot water (~15 gallons) and low foaming caustic cleaner, Klenzade FastPac 110: Mechanical and CIP cleaner (EcoLab, St. Paul, MN), at approximately 2%w/w. The pump was started and the solution was circulated for 8 minutes and then the cleaning solution was drained. The reservoir was again filled with fresh hot water, circulated and then drained. Once rinsed and drained, the reservoir was filled again with fresh hot water and Ster-Bac Quaternary Ammonium Sanitizer (EcoLab, St. Paul, MN) at 200 ppm. Sanitizer was circulated for 2 minutes and then drained. The entire unit was then rinsed again with fresh water. A 50 ml sample was obtained from the rinse water and pour plated on trypticase soy agar (TSA, Difco) to ensure the system is free from microbial contamination.
VITAE

Karol Gailunas, the daughter of Charles and Barbara Gailunas, was born and raised in Catonsville, MD where she attended the Magnet School for Pre-Engineering and Student Conducted Research at Woodlawn High School, graduating in 1997. Following high school, she attended Virginia Polytechnic Institute and State University and received her Bachelor’s degree in Biology and a Minor in Chemistry in 2001.

After graduation, she decided to stay at Virginia Tech to pursue a Master’s degree in Food Science and Technology. She began the program in the summer of 2001. While in graduate school, she was a member of the Institute of Food Technologists and the International Association for Food Protection.