The control, survival, and growth of Listeria monocytogenes on food products

Richelle Lynn Beverly
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THE CONTROL, SURVIVAL, AND GROWTH
OF LISTERIA MONOCYTOGENES
ON FOOD PRODUCTS

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy
in
The Department of Food Science

by
Richelle Lynn Beverly
B.S., Southern University and A&M College, 1997
M.S., The Ohio State University, 1999
December 2004
Dedicated to
My Parents,
Mr. and Mrs. Roosevelt Beverly
ACKNOWLEDGEMENTS

First, I would like to thank God, who is the head of my life. It is with his arms of protection I am able to make it from day to day.

I would like to thank my parents, Mr. and Mrs. Roosevelt Beverly for all of the love, encouragement, prayers and guidance that they have shown me throughout my life. It is because of their total support that I have the strength and courage to pursue my educational and personal goals.

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Last but not least, to my husband Larry D. Richardson, I would like to thank him for his encouragement and support. I would also like to thank him for helping me keep a leveled headed, no matter what insane ideas or plans I managed to conjure up.
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ABSTRACT

Listeria monocytogenes, a ubiquitous foodborne pathogen, was recognized over 70 years ago. It is the source of the human disease listeriosis. The majority of Listeria monocytogenes that have been isolated from food product or human cases are of the serotypes ½ a, ½ b and 4b. Due to the recent outbreaks, recalls and deaths associated with Listeria monocytogenes in ready-to-eat meat products, the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) on October 2, 2003 issued a directive for the control of Listeria monocytogenes on ready-to-eat products. The ready-to-eat food industry must impose a post-lethality treatment and/or growth inhibitor for Listeria monocytogenes on ready-to-eat products. The purpose of this study was to assess the use of different antimicrobial treatments for the inhibition of Listeria monocytogenes on the surface of food products. The utilization of natural ingredients such as cranberries as well as chemicals such as acidified sodium chlorite was employed. An edible film made of chitosan that was dissolved in acetic acid and lactic acid was also evaluated. The survival of Listeria monocytogenes at freezer temperatures on a variety of ready-to-eat meat products was also assessed. Our study has been successful in understanding the survival of Listeria monocytogenes at freezer temperatures on the surface of ready-to-eat meat products under vacuum and non-vacuum package storage conditions. It has also been observed that the use of cranberry juice, acidified sodium chlorite, and chitosan have great potential antimicrobial properties that can be employed by food processors.
CHAPTER 1

INTRODUCTION
The desire to consume healthier foods is a demanding request of consumers. The average consumer tries to improve his/her daily diet due to personal preference or medical reasons. On the a whole, consumers want foods that are low in calories, low in fat, low in sodium and high in fiber, but great in taste. In recent years, the United States general public has also been concerned with the microbial safety and quality of the meat and meat products they purchase. In the 1990’s there were a number of foodborne illness outbreaks occurring in ground beef products and ready-to-eat products. Consumers have demanded that food products have a more natural content rather than chemical content. The safety of meat and meat products not only worries the general public, but is of great concern to persons in the meat industry. Processors use a series of trimming techniques, washing methods, chlorine sprays, organic acid sprays and steam treatment procedures to reduce the microbial load on meat products (Kochevar et al., 1997; Reagan et al., 1996; Cutter and Siragusa, 1995; and Prasai et al., 1995). Still, these various methods have not reduced the numbers of recalls and outbreaks from occurring.

There is a great need to understand the survival and growth of *Listeria monocytogenes* on ready-to-eat meat products. Freezing is a well known method for preservation of food products. This study was conducted to the understanding the survival status of *Listeria monocytogenes* on the surface of a variety of frozen ready-to-eat products. Along with studying the frozen storage conditions, the use of vacuum versus non-vacuum packaging conditions were evaluated.

The use of antimicrobial agents and edible films in food processing are ways in which processors can produce a safer product. The incorporation of these agents into various food product formulas or prepared entrees allows products to be microbial safe.
with the enhanced ability to extend shelf life. Acidified sodium chlorite (ASC) is a product of the acidification of sodium chlorite (NaClO2). ASC works by forming oxychlorous antimicrobial intermediates as it comes into contact with organic matter (Gordon et al., 1972, Kross, 1984). The intermediates products are broad–spectrum germicides that break the oxidative bonds on the cell membrane surface (Kross, 1984, Kemp et al 2000). ASC was evaluated for its effectiveness as a processing aid for dipping or spraying to control \textit{Listeria monocytogenes} on the surface of ready-to-eat products.

The use of chitosan as an edible film on meat products to control bacterial growth has been only evaluated in a small number of studies. Edible films can serve as a barrier to prevent loss of moisture and improve shelf life. Chitosan is derived by the deacetylation of chitin. The antimicrobial action is postulated to be mediated by the electrostatic forces between the protonated amine groups (NH$_2$) in chitosan and the negative residues at cell surfaces (Tsai et al., 1999). Further studies need to be conducted to determine if \textit{Listeria monocytogenes} growth can be inhibited on the surface of ready-to-eat meat products coated with chitosan.

Cranberry juice is a product that contains large amounts of phenolic compounds. Phenolic compounds can inhibit microbial growth of bacterial cells by disputing the cell membrane function (Caldwell, 1995). The use of cranberry juice as a marinade on raw seafood was evaluated.

This dissertation demonstrates the survival status of \textit{Listeria monocytogenes} at freezer temperatures as well the use of new and natural resources and techniques that
processors can employ in their various food formulas to reduce the microbial load on food products.

1.1 REFERENCES


It is estimated that microbial pathogens in foods are the cause for 6.5-33 million cases of human illness and up to 9,000 deaths in the United States each year (Buzby, 2002). Over 40 different foodborne microbial pathogens, including fungi, viruses, parasites, and bacteria, are believed to cause human illnesses. For six bacterial pathogens, *Campylobacter* species, *Salmonella* species, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Escherichia coli* O157 non-O157 STEC, and *Listeria monocytogenes*, the costs of human illnesses are estimated to be $9.3-$12.9 billion annually. Of these costs, $2.9-$6.7 billion is attributed to foodborne bacteria. To estimate medical costs and productivity losses, the Economic Research Services (ERS) uses four severity categories for acute illnesses: those who did not visit a physician, visited a physician, were hospitalized, or died prematurely. The lifetime consequences of chronic disease are included in the cost estimates for *E. coli* O157:H7 and fetal listeriosis. ERS estimates that, each year in the United States, the costs of the acute illness from foodborne *Listeria* are $2.3 billion (Buzby, 1996).

### 2.1 CHARACTERISTICS OF LISTERIA SPECIES

*Listeria* bacteria are short rods measuring 0.4-0.5 μm by 0.5-2.0 μm with rounded ends (Seeliger and Jones, 1986) that are ubiquitous in nature. It is a Gram positive, facultative anaerobic bacterium with both psychrotropic and mesophillic features. *Listeria* species are found in the intracellular state within monocytes and neutrophils (Gray and Killinger, 1966). The bacteria may be curved in single or short chains, often in a “V” shape. *Listeria monocytogenes* is motile due to one to five peritrichous flagella, which may be lost as the bacteria enter the human cell. Movement is still possible because the bacteria polymerize actin into long actin tails that propel the bacteria through the
cytoplasm (Salyers and Whitt, 2002). Filaments, ranging in size of 6-20µm, may develop in old cultures. Older cultures may also stain irregularly. Table 2.1 shows various factors with the upper, lower, and optimal limits that encourage the optimum growth of \textit{Listeria monocytogenes}.

### 2.2 HISTORY AND TAXONOMY OF \textit{LISTERIA} SPECIES

\textit{Listeria monocytogenes} has been labeled as a leading cause of death due to food borne illness (Buzby, 2002; Donnelly, 2001). \textit{Listeria} may have been recognized early in 1891 in tissue samples from German patients. It was isolated from the liver of rabbits in 1911 in Sweden and the disease syndrome recognized in sheep in Germany in 1925 (McCarthy, 1990; Wehr et al., 1987; Gray and Killinger, 1966).

\textit{Listeria}, described as the causative agent of an epizootic in rabbits and guinea pigs in 1924, were small Gram-positive rods named \textit{Bacterium monocytogenes}. The monocytogenes was based on the pronounced mononucleosis evidence in infected rabbits. These same organisms in 1927 were later named Listerella hepatolytica due to the liver marks in infected animals and in honor of Lord Lister (Seeliger, 1961). In 1940, the name was changed to \textit{Listeria monocytogenes} (McCarthy, 1990 and Welshimer, 1981).

The genus \textit{Listeria} contains 6 species: \textit{L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii,} and \textit{L. grayi}. \textit{Listeria monocytogenes} is viewed as the only pathogenic species, but there have been three cases where \textit{Listeria ivanovii} (Busch, 1971; Rocourt and Seeliger, 1985) and one case where \textit{Listeria seeligeri} (Hof and Rocourt, 1992) were reported to cause human infection.
Table 2.1: Growth conditions for *Listeria monocytogenes*.

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<th>Optimum</th>
<th>Upper Limits</th>
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<td>37</td>
<td>45</td>
</tr>
<tr>
<td>pH</td>
<td>4.4</td>
<td>7.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Salt Tolerance (%)</td>
<td>--------------</td>
<td>10</td>
<td>25% at 4°C</td>
</tr>
<tr>
<td>O₂ Availability%</td>
<td>--------------</td>
<td>5</td>
<td>--------------</td>
</tr>
<tr>
<td>Water Activity(Aw)</td>
<td>0.90</td>
<td>0.92</td>
<td>0.97</td>
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</table>
2.3 EMERGENCE OF *LISTERIA MONOCYTOGENES* AS A FOODBORNE PATHOGEN

Changes in food production and demands of a growing society have increased the number of incidences of food borne illness (Oldfield, 2001). A study commissioned by the American Meat Institute (1996) found that societal changes such as more women going to work and more single heads of household caused people to have less time for food purchasing and preparation. The need for convenience has driven the everyday food choices for the past few years. Frozen and ready-to-eat foods accounted for more than one-quarter of dinner entrees (Sloan, 2003b). In 2003, the number one trend in the food industry was the ready-to-eat, heat-and-eat, packaged for on-the-go consumption, and no utensils required (Sloan, 2003a).

*Listeria monocytogenes* has also developed as a foodborne pathogen due to several other factors. One factor is the development of technology, which provides cold storage. This new storage condition provides adequate temperatures for the growth and survival of *Listeria monocytogenes*. A second factor is the achievements and hindrances of the medical community with the increase in the population of elderly, immunocompromised, acquired immune deficiency syndrome (AIDS), transplant and cancer patients. However, one of the main factors for the increase of the emergence of *Listeria monocytogenes* is the production of minimally processed foods, mainly ready-to-eat products, which may or may not be heated to temperatures to properly destroy the pathogen.

2.4 *LISTERIA MONOCYTOGENES* AS A FOODBORNE PATHOGEN

The trait that makes *Listeria monocytogenes* difficult to control during food processing is that it can multiply over a wide range of temperatures, especially at
refrigerator temperatures (Salyers and Whitt, 2002). The classical way of enriching for
Listeria in samples that contain other bacteria is to incubate the sample for prolonged
periods of time in the refrigerator. Some studies suggest that 1-10% of humans may be
intestinal carriers of Listeria monocytogenes without exhibiting signs of illness. Food
and Drug Administration (FDA) and Food Safety and Inspection Service (FSIS) are the
two federal agencies that regulate food in the United States. FSIS regulates meat and
poultry products and processed eggs, while FDA regulates all other food products. At the
end of 2003, FDA reported over 36 recalls (figure 2.1) specifically due to the foodborne
pathogen Listeria monocytogenes (Anonymous, 2003b). In turn FSIS placed 12, class I,
recalls (figure 2.2) on meat and poultry products in 2003 (Anonymous, 2003a). It is
estimated by the Centers for Disease and Control (CDC) that approximately 2,500 cases
of listeriosis are reported each year with 500 of the cases resulting in death (Mead et. al.,
1999). The incidence of Listeria monocytogenes is relatively low, but the consequences
of infection may be severe. Levels of Listeria monocytogenes have been recovered at
greater than 10^3 CFU/g. Foods containing 100 CFU/g or greater should be considered
adulterated (Robert and Greenwood, 2003)

Listeria monocytogenes has been found in at least 37 mammalian species, both
domestic and feral, as well as at least 17 species of birds and possibly some species of
fish and shellfish. Studies have identified sheep as a major reservoir of Listeria in nature
(Rodriguez et al., 1984). Listeria monocytogenes can be isolated from soil, silage, and
other environmental sources. Listeria monocytogenes is quite hardy and resists the
deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does
not form spores. Listeria monocytogenes are pathogenic to varying degrees based on the
Figure 2.1: Recalls reported in 2003 by the Food and Drug Administration (FDA) due to *Listeria monocytogenes* contamination.
Figure 2.2: Recalls reported in 2003 by the Food Safety and Inspection Service (FSIS) due to *Listeria monocytogenes* contamination.
different serotypes (Anonymous, 1992). The production of sulfhydryl-activated hemolysin, listeriolyisin O (α-listeriolysin) is associated with the pathogenic potential of *Listeria monocytogenes* (Geoffroy et al., 1989; Gaillard et al., 1987).

### 2.5 LISTERIOSIS

*Listeria monocytogenes* is the causative agent for the disease Listeriosis.

Listeriosis is a zoonotic illness that affects both animal and humans. Figure 2.3 diagrams the invasive and noninvasive forms of the illness characterizing listeriosis. Due to the long incubation period it is difficult to trace the source of an outbreak.

It is estimated that 5% of healthy humans harbor *Listeria monocytogenes* in their gastrointestinal tract (Donnelly, 2001; Mead et. al, 1999). Humans shed the bacteria in their stools and may show no signs of the illness (Gellin et al., 1989). The Economic Research Services (ERS) estimated that, each year in the United States, the costs of the acute illnesses from foodborne Listeria are $2.3 billion (Buzby, 2002). Nyfeldt (1929) described the first known human case of listeriosis in 1929 (Donnelly, 2001).

The persons affected by Listeriosis are a well-defined high-risk group that includes: pregnant women, neonates and immunocompromised adults. The mortality rate associated with listeriosis is 20% (Slutsker and Schuchat, 1999)

### 2.6 PATHOGENESIS OF *LISTERIA MONOCYTOGENES*

*Listeria monocytogenes* is a highly invasive intracellular pathogen. Macrophages actively ingest *Listeria monocytogenes*. It is this internalization of the bacterium that is triggered by *Listeria monocytogenes*. The pathogen appears in a vacuole, which is later lysed by the bacteria allowing it to escape into the cytoplasm of the host cell. The bacterium then polymerizes actin filaments at one end to form long actin tails that propel
it through the cytoplasm (Salyers and Whitt, 2002). The adherence and invasion are mediated by membrane proteins “cal internalins”: InlA and InlB.

The forced phagocytosis brings *Listeria monocytogenes* into the host cells encased in a vacuole. The bacteria then escape the vesicle by a protein hemolysin, listeriolysin O (LLO) (Salyers and Whitt, 2002; Kuhn and Goebel, 1999). LLO is a pore-forming cytotoxin. It is also responsible for the zone of beta-hemolysis seen on blood agar plates. The gene encoding LLO is named “hly” for “hemolysin.”

The virulence genes of *Listeria monocytogenes* whose products are involved in the intracellular life cycle are a cluster on the chromosome and called the PrfA-dependent virulence gene cluster. The cluster comprises six genes, *prfA, plcA, hly, mpl, actA, plcB* (Kuhn and Goebel, 1999).

### 2.7 METHODS TO INHIBIT THE GROWTH OF *LISTERIA MONOCYTOGENES*

To control postproduction contamination of food products, many avenues of research have been explored. The use of hazard analysis and critical control point (HACCP) programs has allowed processors to identify areas of concern and address the problem. Other methods for the reduction of *Listeria monocytogenes* include the use of low dosage irradiation (IFST, 1999; Monk et al., 1995) and high dosage irradiation (Foong et al., 2004). *Listeria monocytogenes* has also been inhibited in juice concentrates (Nogueira et al., 2003), thermal inactivation in broth (Jesús and Whiting, 2003) and through pH enhancement with ammonia gas on beef trimmings (Niebuhr and Dickerson, 2003). The decontamination of meat includes, but is not limited to, rinsing with water and chemical such as acetic acid and lactic acid (Siragusa, 1995).
Adapted from Donnelly, 2001

**Figure 2.3:** Characterization of the invasive and noninvasive syndromes associated with adult Listeriosis.
2.8 SEROLOGICAL ASSOCIATION OF *LISTERIA SPECIES*

Serology is a classic tool for epidemiological studies. It is the analysis of the properties and effects of serums (blood, semen, saliva, sweat, or fecal matter) to detect the presence of antibodies against a microorganism. *Listeria* species strains are serotyped based on the cellular (O) and flagella (H) antigens (Graves et al., 1999; Seeliger and Hohne, 1979). The serology description of *Listeria* is described by Seeglier and Donker based on 15 ‘O’ antigens and 5 ‘H’ antigens (Lovett, 1990). Table 2.2 provides a list of the serovars of *Listeria* species. The 4bX serotype is a variant of 4b with implication to an outbreak due to pâté in the United Kingdom (Graves et al., 1999).

Serology is useful when the epidemiology is crucial to an outbreak or a case study. Table 2.3 lists the various serotypes of the *Listeria* species. Over 90% of *Listeria monocytogenes* isolates can be serotyped with commercially available sera. Sera is serum which contains antibodies that have been obtained from an animal which has been immunized either by antigen injection or infection with microorganisms containing the antigen. Most *Listeria monocytogenes* isolates obtained from patients and the environment are type 1 or 4 (Hitchins, 2003). All nonpathogenic species, except *Listeria welshimeri*, share one or more somatic antigens with *Listeria monocytogenes*. It is due to this reason that the FDA suggests that serotyping alone without the completion of identification procedures is inadequate for identification of *Listeria monocytogenes*.

*Listeria monocytogenes* is the only species that causes infections in humans while *Listeria ivanovii* is sporadically associated with abortions in animals. Greater than 95% of human infections caused by strains of *Listeria monocytogenes* belong to the serotype
Table 2.2: Serovars of the genus *Listeria*.

<table>
<thead>
<tr>
<th>Seeliger and Donker-Voet Designation</th>
<th>O antigens (Somatic)</th>
<th>H antigens (Flagella)</th>
</tr>
</thead>
<tbody>
<tr>
<td>½ a</td>
<td>I, II, (III)(^a)</td>
<td>A B</td>
</tr>
<tr>
<td>½ b</td>
<td>I, II, (III)(^a)</td>
<td>A B C</td>
</tr>
<tr>
<td>½ c</td>
<td>II, (III)(^a)</td>
<td>B D</td>
</tr>
<tr>
<td>3a</td>
<td>II, (III), IV</td>
<td>A B</td>
</tr>
<tr>
<td>3b</td>
<td>(III), IV(^a), (XII)(^a), (XIII)(^a)</td>
<td>A B C</td>
</tr>
<tr>
<td>3c</td>
<td>(III)(^a), IV(^a), (XII)(^a), (XIII)(^a)</td>
<td>B D</td>
</tr>
<tr>
<td>4a</td>
<td>(III)(^a), (V)(^a), VI, VII, VIII(^b), IX, X(^b), XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>4ab</td>
<td>(III)(^a), V, VI, VII(^b), VIII(^b), IX(^b), X(^b), XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>4b</td>
<td>(III)(^a), V, VI, VII(^b), VIII(^b), IX(^b), X(^b), XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>4c</td>
<td>(III)(^a), V, VI, VII, VIII(^b), IX(^b), X(^b), XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>4d</td>
<td>(III)(^a), V, VI, VII, VIII(^b), IX(^b), X(^b), XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>4e</td>
<td>(III)(^a), V, VI, VII(^b), VIII(^a), (IX)(^a)</td>
<td>A BC</td>
</tr>
<tr>
<td>5</td>
<td>(III), V, VI, VII(^b), VIII(^b), IX(^b), X, XI(^b), XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>6a</td>
<td>(III)(^a), V, VI, VII, VIII(^b), IX, X(^b), XI(^b), XV</td>
<td>A B C</td>
</tr>
<tr>
<td>6b</td>
<td>(III)(^a), V, VI, VII, VIII(^b), IX, X, XI, XV(^b)</td>
<td>A BC</td>
</tr>
<tr>
<td>7</td>
<td>(III)(^a), XII(^a), XIII(^a)</td>
<td>A B C</td>
</tr>
</tbody>
</table>

( ) means antigen not always present
\(^a\) O antigen reported by Lovett, 1990 and not Graves et al., 1999
\(^b\) O antigen reported by Graves et al., 1999 and not Lovett, 1990
\(^c\) Identified from outbreak in United Kingdom (McLauchlin et al., 1991)

Adapted from Lovett, 1990; Graves et al., 1999
Table 2.3:  Serotypes of the *Listeria* species.

<table>
<thead>
<tr>
<th>Listeria species</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>½ a, ½ b, ½ c 3a, 3b, 3c 4a, 4ab, 4b, 4c, 4d, 4e, “7”</td>
</tr>
<tr>
<td><em>Listeria ivanovii</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Listeria innocua</em></td>
<td>4ab, 6ab, 6b, Un(^a)</td>
</tr>
<tr>
<td><em>Listeria welshimeri</em></td>
<td>6a, 6b</td>
</tr>
<tr>
<td><em>Listeria seeligeri</em></td>
<td>1/2b, 4c, 4d, 6b, Un</td>
</tr>
</tbody>
</table>

\(^a\) Un, undefined

Adapted from Hitchins, 2003
½ a, ½ b, and 4b (Graves et al., 1999). These same serotypes of *Listeria monocytogenes* are associated with the major foodborne outbreaks.

### 2.9 DETECTION AND RAPID IDENTIFICATION OF *LISTERIA* SPECIES

Due to a regulation passed by the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) on October 2, 2003 for the control of *Listeria monocytogenes* on ready-to-eat products, the RTE food industry must find ways to identify *Listeria* species in their plants, while continuing to manufacture, warehouse, and sell product in a timely manner to make a profit. It is due to the “Profit Factor” that processors are looking for rapid detection procedures.

Proper identification of *Listeria* species must be made because both *Listeria monocytogenes* and *Listeria innocua* are found in food products. The standard rule for detection and enumeration of *Listeria monocytogenes* is outlined in the Bacteriological Analytical Manual (BAM). The procedure involves an enrichment step of a 25-gram sample in an enrichment broth with the addition of selective agents after 4 hours. The broth is later reincubated at 30°C for a complete enrichment time of 48 hours. The enrichment broth is then streaked at 24 and 48 hours on differentia selective agars to isolate the *Listeria* species (Hitchins, 2003).

Companies have recently developed methods to identify *Listeria monocytogenes* within a 24 hour to 3 day time period. API *Listeria* strips manufactured by the bioMérieux (France) can identify *Listeria* species. These strips work on the principle of how the suspected sample reacts based on sugar fermentation and enzymatic reactions. Based on the color reaction, a number code identifies the specific species. Oxoid
(Hampshire, England) has developed a new Chromogenic Listeria agar plate that turns presumptive *Listeria* species colonies blue and presumptive *Listeria monocytogenes* colonies blue with an opaque halo. Oxoid also detects *Listeria* species with a Listeria Rapid Test that uses anti-flagellin antigens with blue latex to test for the ‘B’ flagella antigen found on *Listeria* species. The O.B.I.S. Mono test from Oxoid has a test card, which uses a suspect colony and gives results in 10 minutes. The Vedas® system is an automated system by bioMérieux (France) which runs 60 different tests on the sample in 45 minutes based on the enzyme link immunoassay (ELSIA) principle. Other rapid tests are immunoprecipitate detection, VIP® by Biocontrol and Singlepath® by Merck, polymerase chain reaction (PCR), BAX® by DuPont, and Biochemical test strips, MicroID by Remel.

Polymerase Chain Reaction (PCR) is a technique used for the rapid, specific and highly sensitive detection of pathogens in food (Somer Kashi, 2003; Hill, 1996). The PCR process consists of a series of twenty to thirty cycles. Each cycle consists of three steps: denaturation, annealing, and extension.

During the implementation of most standard plate count methods for isolating and identifying *Listeria monocytogenes*, the media most often used is a Modified Oxford (MOX) media. Figure 2.4 illustrates the appearance of *Listeria monocytogenes* on modified oxford plates. The modified oxford agar is based on the formula of Curtis et al. (1989) for isolation of *Listeria monocytogenes*. This media contains a selected peptone formula that provides nitrogen, vitamins and minerals. Cornstarch is omitted to reduce opalescence. Sodium chloride is added to ensure the correct osmotic balance. The addition of lithium chloride and antibiotics, like acriflavin, colistin sulfate, cefotetan,
Figure 2.4: The appearance of *Listeria monocytogenes* on a modified oxford plate after 48 hour of incubation at 37°C.
cycloheximide and fosfomycin, aide in the suppression of growth for Gram-negative bacteria and most Gram-positive bacteria. Lithium chloride gives the medium selectivity due to the high salt tolerance of *Listeria* species. The other antibiotics are derived from the Oxford Listeria Selective Supplement inhibitor (Fluka 75806). Certain strains of *Staphylococci* may grow as esculin negative colonies. Esculin, a product of a coumarin derivative extracted from the bark of flowering ash (Fraxinus ornus), is a glycoside decomposed of glucose and a dihydroxycoumarin compound. *Listeria monocytogenes* hydrolyzes esculin to esculetin and forms a black complex with iron (III) ions. Therefore *Listeria monocytogenes* forms brown-green colored colonies with a black halo.

### 2.10 REGULATION ON *LISTERIA MONOCYTOGENES*

In June of 2003, FSIS introduced an interim Listeria rule concerning ready-to-eat meat and poultry products in which the ready-to-eat food industry must impose a post-lethality treatment and/or growth inhibitor for *Listeria monocytogenes* or employ sanitation measures for control of *Listeria monocytogenes* in the processing environment. This rule was later revised on October 2, 2003. The United States Department of Agriculture, Food Safety and Inspection Service issued a new directive for the control of *Listeria monocytogenes* in Ready-to-Eat (RTE) products. The new directive is for “Verification Procedures for the *Listeria monocytogenes* Regulation and Microbial Sampling of Ready-to-Eat (RTE) Products for the FSIS Verification Testing Program.”

### 2.11 READY-TO-EAT FOOD PRODUCTS

The Food Safety Inspection Service (FSIS) defines a Ready-to-Eat (RTE) product as a meat or poultry product that is in a form that is edible without additional preparation
to achieve food safety and may receive additional preparation for palatability or aesthetic, epicurean, gastronomic, or culinary purposes. RTE products are not required to bear safe-handling instructions (as required for non-RTE products by 9 CFR 317.2(1) and 381.125 (b)) or other labeling that directs that the product must be cooked or otherwise treated for safety, and can include frozen meat and poultry products (FSIS Directive 10,240.4, 2003) (Food Safety and Inspection Service, 2003). RTE products fall under the label of high-risk foods. Under the label of high risk foods are: (1) foods stored at refrigerator temperatures for a long period of time, (2) raw produce fertilized with manure with no kill step, and (3) ready-to-eat (RTE) foods contaminated after being processed and not reheated to high enough temperature.

2.12 CROSS CONTAMINATION OF READY-TO-EAT PRODUCTS

A number of factors can cause or contribute to the contamination of ready-to-eat meat and poultry products in a meat or poultry processing establishment by *Listeria monocytogenes*. Figure 2.5 illustrates a flow diagram of cross-infection and cross-contamination that begins with the farm livestock. The first hurdle that a processor must handle is if the pathogen is already present. It may be in the product ingredients, if so, a processing error, such as incorrect formulation or inadequate processing time or temperature, can result in the production of products containing live organisms. The second obstacle is that a product that has undergone a successful lethality treatment can be contaminated by biofilms on food-contact surfaces of equipment used for processing, handling, or packaging the product. The product can also be exposed to environmental contamination or cross-contamination in the post-lethality processing environment.
Cross-contamination can come in the form of plant construction in the post-lethality area of the establishment. Precautionary measures or steps during facilities construction or remodeling must be taken to protect the products during construction or serious outbreaks of listeriosis may occur. Figure 2.6 helps to illustrate the factors that influence the human exposure to *Listeria monocytogenes* from ready-to-eat products. Additional causes of contamination or cross-contamination can be poor facilities design or plant equipment layout where the flow paths of raw product and finished products cross or if vehicle or personnel traffic from outside the plant or from a raw-product area of the plant enters an area where exposed finished products are handled. Contamination or cross-contamination also can occur if processing equipment has not been designed for easy cleaning, or if equipment or facilities have hard-to-reach niches that can harbor *Listeria monocytogenes* or other pathogens (Federal Register, 2003).

### 2.13 ANTIMICROBIAL AGENTS

In the processing of ready-to-eat products, the use of antimicrobial agents is a common practice. Antimicrobial agents are substances in or added to an RTE product that has the effect of reducing or eliminating a microorganism, or that has the effect of suppressing or limiting growth in the product throughout the shelf life of the product (FSIS Directive 10,240.4, 2003). The purpose of antimicrobial agents is to reduce or eliminate microbial activity in two different manners: stasis or cidal mechanisms. Agents that inhibit microbes are said to be static. Cidal antimicrobial agents destroy or kill microbes. Many chemicals used for in sterilization and disinfections are cidal, killing microbes. However, food preservatives must not be toxic to the consumer and so then tend to be static agents.
Figure 2.5: Pathways of cross-infection and cross-contamination. Source: Linton and Hinton. (1987)
Figure 2.6: Factors that affect human exposure to *Listeria monocytogenes* in ready-to-eat products

Adapted from Anonymous, 2000
2.14 REFERENCES


CHAPTER 3

EFFECT OF FROZEN STORAGE TEMPERATURES ON VARIOUS READY-TO-EAT MEAT PRODUCTS INOCULATED WITH *LISTERIA MONOCYTOGENES* THAT ARE VACUUM AND NON-VACUUM PACKAGED
3.1 INTRODUCTION

*Listeria monocytogenes* is a Gram-positive rod that has the capability to grow at refrigerator temperatures. *Listeria* is considered a foodborne pathogen that is the causative agent of Listeriosis. According to the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) in 1999, *Listeria monocytogenes* was responsible for 31 of the 62 (50%) recalls of cooked meat. An outbreak in 1998 and 1999 due to hotdogs/frankfurter resulted in 21 fatalities and approximately 100 reported cases of listeriosis (CDC, 1999). Later in 2002, an outbreak from the consumption of sliced turkey deli meat resulted in 10 outbreaks in the Northeastern United States (CDC, 2000).

Various studies have shown that *Listeria monocytogenes* survives and may increase by 0.5 to 3.0 log units in meat during refrigerated storage (Ikeda et al., 2003; Barbosa et al., 1995; Grau et al., 1992; Grau et al., 1990; Gouet et al., 1978). Porto et al. (2004) surveyed consumers about their storage and preparing practices for frankfurters. Of the consumers surveyed, 71% thought that frankfurters needed to be cooked while 29% thought that no cooking was needed. The proper cooking process for ready-to-eat (RTE) meat products is sufficient for the elimination of bacterial contamination (Roering et al., 1998; Yen et al., 1991; Boyle et al., 1990). Heating to a minimum surface temperature of 70°C for 2 minutes will achieve a 5 log unit reduction of *Listeria monocytogenes* on frankfurters regardless of formulation or prior storage temperatures (Porto et al., 2004). However, bacterial contamination of finished products may be due to inadequate heating during processing or post-production contamination (McKellar et al., 1994; Yousef et al., 1991; Wenger et al., 1990).
Listeria monocytogenes is a foodborne pathogen with a zero tolerance policy that results in nearly half of the deaths from foodborne pathogen infections (Donnelly, 2001). Nevertheless, despite the efforts of government agencies and processors, studies have shown that Listeria monocytogenes is capable of survival at freezer temperatures in products such as broth (El-Kest et al., 1991), milk, ice cream, (Anonymous, 1987; Anonymous, 1986,) and yogurt (Flahenty, 1989).

Freezing is a preservation technique that extends a product’s shelf-life. This process lowers the temperature to levels in which metabolic processes are stopped and the rates of chemical and biochemical reactions reduced. During the freezing process, intracellular and extracellular ice crystals form which causes death and injury to microbial cells. Freezing has been observed to have lethal and sublethal effects on Listeria monocytogenes based on the speed of freezing, temperature of frozen storage, cycles of freezing and thawing and the presence of nutrients in the freezing media (El-Kest et al., 1991). Freezing increases the sensitivity of Listeria monocytogenes to lysozymes and lipases which occur naturally in some foods (Lou and Yousef, 1999).

Studies have shown that Listeria monocytogenes can be isolated from ice cream (Anonymous, 1986; Anonymous, 1987) and frozen yogurt (Flahenty, 1989). El-Kest and Marth (1991) reported that studies done by Keith (1913) states that milk and ice cream favor the survival of bacteria at low temperatures because they encompass the physical conditions that are protective to bacteria. Milk components, such as lactose, casein, and milk fat provide protection for Listeria monocytogenes against death and injury during long term storage (El-Kest and Marth, 1991).
A survey by the American Meat Institute Foundation reveals that 15% of people admit to eating hotdogs/frankfurters without reheating before consumption. Our study observed the effects that freezing had on the survival status of *Listeria monocytogenes* on ready-to-eat meat products that are under vacuum and non-vacuum packaged conditions.

### 3.2 MATERIALS AND METHODS

#### 3.2.1 Culture Growth Conditions

*Listeria monocytogenes* strain V7 (serotype ½ a) obtained from the Centers for Disease Control (CDC), Atlanta, GA., U.S.A., that was originally isolated from the blood of an infected individual, was used during this study. The *Listeria monocytogenes* culture was grown for 18 hours in Brain Heart Infusion Broth (BHI) (Difco, Detroit, Mich., U.S.A.) at 37°C. The pure cultures were stored at -70°C and subcultured twice in BHI Broth at 37°C for 24 h before being used.

#### 3.2.2 Types of Ready-to-Eat Products

The turkey product was labeled as a fully cooked, boneless smoked white turkey. The ingredients were: white turkey, water, salt, dextrose, modified food starch, sodium phosphate, carrageenan, sodium erythorabte, and sodium nitrite. The hot dog wieners were labeled as fully cooked, artificially colored, hickory smoke flavoring added and made with turkey, pork, and beef. The ingredient label listed: mechanically separated turkey, pork, corn syrup, water, beef, salt, and not more than 2% potassium lactate, dextrose, flavorings, extractives of paprika, sodium phosphate, sodium diacetate, sodium erythorbate, smoke flavoring, sodium nitrite, paprika and FD&C Red #40. The smoked sausage was a pork product that contained: pork, water salt corn syrup, dextrose, sugar, sodium phosphate, paprika, flavorings, sodium erythorbate, autolyzed yeast extract, and
sodium nitrite. The roast beef was labeled as fully cooked, containing water, isolated soy protein, carmel color, hydrolyzed corn protein, flavoring and spice. The balance of the ingredient label list: beef, water, dextrose, isolated soy protein, salt potassium lactate, modified food starch, flavorings, sodium, phosphate, carmel color, and spice.

3.2.3 Inoculation of Ready-to-Eat Products

The ready-to-eat products for this study were roast beef, hot dogs, pork sausage, chicken breast, and deli style turkey breast. The meat samples were cut into 5gram cubes using aseptic techniques. An overnight *Listeria monocytogenes* culture was decimally diluted to 6.0 logs CFU/g. One milliliter of the *Listeria monocytogenes* culture was inoculated onto each of the cubed meat samples. The culture was allowed to air dry on the cube meat samples for 2 minutes under a laminar flow hood. The samples were then vacuum packaged (Food Safer7, Tilia International Inc., San Francisco, CA) or non-vacuum packaged (Whirl-Pack® bags) with label. The samples were frozen at -20°C and bacterial counts determined at day 0, 7, 14, 21, 28, 30, 60 and 90 by adding 5 ml of phosphate buffer saline (PBS) solution into each bag, stomaching for 1 min, making serial dilutions and plating the dilutions onto modified Oxford agar (Acumedia, Baltimore, MD, U.S.A.) with a selective supplement (cycloheximide, colistin sulphate, acriflavine, cefoyetan and fosfomycin, each at 20 mg/L) (Oxoid, Hampshire, England). The plates were incubated at 37°C for 48 hours and colony counts expressed in log CFU/g. The only bacteria present on the media were the added cells of *Listeria monocytogenes* strain V7. This was confirmed by plating the control non-inoculated cubed of each meat sample to ensure no other *Listeria* species was present.
3.2.4 Statistical Analysis

The effects of the -20°C frozen storage of the ready-to-eat products inoculated with *Listeria monocytogenes* strain V7 on the surface were analyzed by statistical comparisons of all pairs using one-way analysis of the variance (ANOVA) (JUMPIn version 4.0.3, SAS Institute Inc., Cary, N.C., U.S.A.). Statistical significance occurs at P<0.05. All experiments were repeated 3 times.

3.3 RESULTS AND DISCUSSION

The meat products used in this study were purchased at a local grocer except for the chicken. The chicken breast was supplied by a poultry processor. The turkey deli meat and the hot dog wieners (Anonymous, 2004) were selected due to the various recalls and outbreaks associated with the two products. The sausage (FSIS, 2002a; FSIS, 2002b), roast beef (FSIS, 2002c) and chicken (FSIS, 2003a; FSIS, 2003b) products were chosen because of their capacity to be considered as a ready-to-eat product by consumers.

The hot dogs were the only product to state on the label to “Always Cook Thoroughly.” While the turkey product serving suggestions were “Turkey is fully cooked and can be served hot or cold.” There were no cooking instructions or serving suggestions on the sausage. All the products stated that they must be refrigerated.

The chicken was cubed chicken breast that was boiled in bag until fully cooked. The chicken was selected to view the growth of *Listeria monocytogenes* on an addition poultry product without the addition of ingredients to reduce microbial contamination. Each product tested had some type of ingredient added that could inhibit microbial growth except for the chicken breast.
Figure 3.1 shows the survival of *Listeria monocytogenes* after 28 days of storage at -20°C under vacuum and non-vacuum packaging conditions of ready-to-eat roast beef. It is seen that during the first 14 days that the counts increase regardless to if the roast beef is vacuum or non-vacuum packaged. However, by day 28, the *Listeria monocytogenes* counts significantly increased to 0.8 log CFU/g compared to day 0. Then the *Listeria monocytogenes* counts were significantly decrease by day 60 and 90 for the vacuum and non-vacuum roast beef samples (Figure 3.2). There was no significant difference between the *Listeria monocytogenes* counts on day 90 when compared to counts on day 0 for both vacuum and non-vacuum packaging roast beef. Furthermore, there was no significant difference in *Listeria monocytogenes* counts between the vacuum or non-vacuum samples throughout the 90 day study (Figure 3.2).

Figure 3.3 shows the recovery of *Listeria monocytogenes* on the surface of ready-to-eat hotdogs after storage at -20°C under non-vacuum and vacuum packaging conditions for 28 days. From day 14 to day 28, *Listeria monocytogenes* counts on the surface of vacuum packaged hotdogs were significantly higher compared to day 0. Whereas for the non-vacuum hotdogs the *Listeria monocytogenes* counts had also significantly increased from day 14 to 28. However figure 3.4 shows that the vacuum packaged hotdogs had significantly higher counts than the non-vacuum packaged hotdogs. Nevertheless, by day 90 the non-vacuum hotdogs had dropped to the initial inoculation levels and the vacuum hotdogs were only 0.2 log CFU/g higher from the initial inoculation levels, which was not a significant change from the initial levels. There was a significant difference between the vacuum packaged hotdogs and the non-
Figure 3.1: The recovery of *Listeria monocytogenes* on ready-to-eat roast beef after storage at -20°C under non-vacuum (a) and vacuum (b) packaging conditions for 28 days.
Figure 3.2: The recovery of *Listeria monocytogenes* on ready-to-eat roast beef after storage at -20°C under vacuum and non-vacuum packaging conditions for 90 days.
Figure 3.3: The recovery of *Listeria monocytogenes* on ready-to-eat hotdogs after storage at -20°C under non-vacuum (a) and vacuum (b) packaging conditions for 28 days.
Figure 3.4: The recovery of *Listeria monocytogenes* on ready-to-eat hotdogs after storage at -20°C under vacuum and non-vacuum packaging conditions for 90 days.
vacuum packaged hotdogs on day 30 and 60. However, by day 90 there was no significant difference between the two packaging conditions (Figure 3.5).

Figure 3.5 shows the recovery of *Listeria monocytogenes* on the surface of ready-to-eat pork smoked sausage after storage at -20°C under non-vacuum and vacuum packaging conditions for 28 days. For both the vacuum and non-vacuum packaged sausage samples, the *Listeria monocytogenes* counts had a significantly increased by 0.6 log CFU/g on day 30 from initial inoculation levels. Figure 3.6 illustrates the recovery of *Listeria monocytogenes* on the surface of ready-to-eat pork smoked sausage after storage at -20°C under non-vacuum and vacuum packaging conditions for 90 days. By day 90, the vacuum packaged sausage *Listeria monocytogenes* counts had dropped below the initial inoculation level. From day 30 to 90, the vacuumed package sausage had significantly lower *Listeria monocytogenes* counts compared to the non-vacuumed packaged sausage, but the non-vacuumed package sausage was reaching the initial *Listeria monocytogenes* counts at day 90 (Figure 3.6).

Figure 3.7 shows the recovery of *Listeria monocytogenes* on the surface of ready-to-eat chicken breast after storage at -20°C under non-vacuum and vacuum packaging conditions for 28 days. From day 14 to 28, *Listeria monocytogenes* counts for both vacuum packaged and non-vacuum packaged chicken were significantly higher compared to day 0. However, there was a significant difference between the non-vacuum packaged chicken and the vacuum packaged chicken (Figure 3.8). In the non-vacuum packaged chicken, *Listeria monocytogenes* counts remained at a constantly lower level in comparison to the vacuum packaged chicken.
Figure 3.5: The recovery of *Listeria monocytogenes* on ready-to-eat pork smoked sausage after storage at -20°C under non-vacuum (a) and vacuum (b) packaging conditions for 28 days.
Figure 3.6: The recovery of *Listeria monocytogenes* on ready-to-eat pork smoked sausage after storage at -20°C under vacuum and non-vacuum packaging conditions for 90 days.
Figure 3.9 shows the recovery of *Listeria monocytogenes* on the surface ready-to-eat smoked white deli turkey after storage at -20°C under non-vacuum and vacuum packaging conditions for 28 days. The vacuumed packaged turkey was the only ready-to-eat meat product that did not have an increase in *Listeria monocytogenes* counts under frozen storage conditions. At day 30, the vacuum packaged turkey samples had decreased in *Listeria monocytogenes* counts by 0.8 log compared to initial levels (Figure 3.10). The vacuum packaged turkey *Listeria monocytogenes* counts reached the initial levels by day 90. The growth for the non-vacuum turkey samples were at the same level for day 90 as for day 0. Throughout the 90 day study, the vacuumed packaged turkey had significantly lower *Listeria monocytogenes* counts compared to non-vacuumed packaged turkey.

*Listeria monocytogenes* has been shown to be capable of maintaining a survival status in various ready-to-eat products that contain ingredients, such as potassium lactate, sodium phosphate, sodium diacetate, and sodium nitrite, commonly used to hinder microbial growth (Porto et al., 2002; Yen et al., 1992). For the three month study of *Listeria monocytogenes*, regardless of the packaging conditions, the type of meat, or the days in storage, *Listeria monocytogenes* was present on the sample and was capable to maintain survival status. Our study has shown that the critical time for *Listeria monocytogenes* to grow in ready-to-eat meat products is the first 30 days of storage at freezer temperatures. It is during this time that log counts can increase by 0.2 to 0.8 log CFU/g depending upon the product or package conditions. Our results differ from previous studies that showed *Listeria monocytogenes* counts remained relatively constant after 1 month of storage at -18°C for pork/beef frankfurters (Porto et al., 2004).
Figure 3.7: The recovery of *Listeria monocytogenes* on ready-to-eat chicken breast after storage at -20°C under non-vacuum (a) and vacuum (b) packaging conditions for 28 days.
Figure 3.8: The recovery of *Listeria monocytogenes* on ready-to-eat chicken breast after storage at -20°C under vacuum and non-vacuum packaging conditions for 90 days.
Figure 3.9: The recovery of *Listeria monocytogenes* on ready-to-eat smoked white deli turkey after storage at -20°C under non-vacuum (a) and vacuum (b) packaging conditions for 28 days.
Figure 3.10: The recovery of *Listeria monocytogenes* on ready-to-eat smoked white deli turkey after storage at -20°C under vacuum and non-vacuum packaging conditions for 90 days.
El-kest and Marth (1991) found that glycerol, at levels as low as 2%, served as a cryoprotective agent in phosphate buffer solution (PBS) from 1 hour to 6 months of frozen storage at -18°C for *Listeria monocytogenes*. Glycerol is a known cryoprotective substance to protect bacteria cultures during frozen storage. It was reported by El-kest and Marth (1991) that the presence of glycerol around bacterial cells allows freezing to happen so that bacteria and other nonaqueous matter are extruded from water crystals without being crushed based on finding by Keith (1913). Milk fat at 2 and 4% also displayed cryoprotective properties for *Listeria monocytogenes* (El-kest and Marth, 1991).

Table 3.1 shows the amount of fat in ready-to-eat products based on product label nutrition facts. It is noted that the ready-to-eat roast beef and turkey have the same amount of fat in them per serving size. However, the ready-to-eat roast beef was a product that contained intramuscular fat, while the fat in the ready-to-eat turkey is mixed throughout the product. Based on the findings by El-kest and Marth (1991) it is hypothesized that the degree of intramuscular fat found in roast beef, the amount of fat in sausage and hotdogs are acting as cryoprotective agents for *Listeria monocytogenes* due to glycerol being the backbone of lipid molecules and the large percentage of fat.

### 3.4 CONCLUSION

*Listeria monocytogenes* is a foodborne pathogen with zero tolerance by regulatory agencies and a major problem for food processors. The effects of freezing to control *Listeria monocytogenes* varies depending on the product and packaging conditions. It is also evident that *Listeria monocytogenes* has the capability of surviving at a temperature of -20°C. These findings are important for food processors when developing and
maintaining their Hazard Analysis and Critical Control Point (HACCP) plans. The findings are also important for the training or retraining of consumers about food safety issues in the home. These findings make it necessary for the development of rapid methods to detect *Listeria monocytogenes* directly from food samples. On a whole, *Listeria monocytogenes* is a foodborne pathogen that has the ability to survive freezer temperatures so consumers should remember that meats need to be properly heated according to manufacture’s instructions to destroy the pathogen.

### 3.5 REFERENCES


Table 3.1: The amount of fat in ready-to-eat products based on product label nutrition facts.

<table>
<thead>
<tr>
<th>Type of Meat</th>
<th>Serving Size (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roast Beef</td>
<td>84</td>
<td>2</td>
</tr>
<tr>
<td>Pork Sausage</td>
<td>56</td>
<td>12</td>
</tr>
<tr>
<td>Smoked White Deli Turkey</td>
<td>56</td>
<td>2</td>
</tr>
<tr>
<td>Chicken Breast</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Hotdog</td>
<td>33</td>
<td>10</td>
</tr>
</tbody>
</table>


CHAPTER 4

ACIDIFIED SODIUM CHLORITE TREATMENT
FOR INHIBITION OF \textit{LISTERIA MONOCYTOGENES} GROWTH
ON THE SURFACE OF READY-TO-EAT ROAST BEEF
4.1 INTRODUCTION

*Listeria monocytogenes*, a Gram-positive facultative, highly motile rod, is an ambiguous foodborne pathogen. *Listeria monocytogenes* is the causative agent behind the disease Listeriosis. There is evidence that suggest *Listeria monocytogenes* is a transitory resident of the intestinal tract in humans with 2 to 10 % of the general population being carriers of the organism without any apparent adverse consequences (Anonymous, 2000). It is estimated by the Centers for Disease Control and Prevention (CDC) that up to 2,5000 cases of listeriosis occur each year with 500 of these cases resulting in death (Mead et al, 1999). The United State Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) suggest that as much as 5% of many ready-to-eat (RTE) foods are contaminated with *Listeria monocytogenes* (Gombas et al, 2002, Hitchins, 1996 and Levine et al., 2001).

The main concern of the food industry is that *Listeria monocytogenes* can grow at refrigerator temperatures and is resistant to various environmental conditions, allowing it to survive longer under adverse conditions. Many efforts have been made to eradicate the organism for ready-to-eat foods (Tompkin, 2002 and Tompkin et al., 1999). The implementation of Hazard Analysis Critical Control Points (HACCP) for food processors has allowed for careful evaluation of processed foods. The identification of critical control points (CCPs) has caused processors to find ways to eliminate the need for additional CCPs. The cooking step in the manufacturing of a ready-to-eat (RTE) meat product is a major CCP. Post-cooking microbial contamination of RTE meats is a serious threat that many meat processors are trying to combat. Methods need to be developed to
control the growth of this pathogen on these products to prevent the economic loss to the food industry.

In June, of 2003, the Food Safety and Inspection Service (FSIS) introduced an interim *Listeria* rule concerning ready-to-eat meat and poultry products in which the ready-to-eat food industry must impose a post-lethality treatment and/or growth inhibitor for *Listeria monocytogenes* or employ sanitation measures for control of *Listeria monocytogenes* in the processing environment. Processors are looking for a number of ways to decrease post-cooking contamination. This rule was later revised on October 2, 2003. The United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) issued a new directive for the control of *Listeria monocytogenes* in Ready-to-Eat (RTE) Products. The new directive is for the “Verification Procedures for the *Listeria monocytogenes* Regulation and Microbial Sampling of Ready-to-Eat (RTE) Products for the FSIS Verification Testing Program.” Processors are looking for a number of ways to decrease post-cooking contamination. One possibility would be the use of acidified sodium chlorite (ASC) solutions that have been approved by the Food and Drug Administration as an antimicrobial agent on processed, comminuted or formed meat food products (21 CFR part 173.325).

Kemp et al., (2000) demonstrated that using an ASC solution as a 5 second dip pre-wash at concentrations of 500 ppm, 850 ppm and 1,200 ppm produced significant reductions in the natural populations of total aerobes on broiler carcasses. There was a reduction of at least 99.4% for *Escherichia coli* regardless of the ASC concentration.

Acidified sodium chlorite is a product of sodium chlorite (NaClO₂) acidification. Once ASC comes into contact with organic matter oxychlorous antimicrobial
intermediates are formed (Gordon et al., 1972, Kross, 1984). The intermediates are broad–spectrum germicides that break the oxidative bonds on the cell membrane surface (Kross, 1984, Kemp et al 2000). The fundamental nonspecific oxidative mode of action of this chemistry is thought to also minimize the potential problem of acquired resistance that often arises in bacterial population following prolonged exposure to antimicrobial procedures (Kemp et al 2000). Once formed, chlorous acid gradually decomposes to form chlorate ion, chlorine dioxide and chloride ion. Its antimicrobial efficacy derives from the uncharged chlorous acid, which is able to penetrate bacterial cell walls and disrupt protein synthesis by virtue of its reaction with sulfhydryl, sulfide and disulfide containing amino acids and nucleotides. The undissociated acid is thought to facilitate proton leakage into cells and thereby increase energy output of the cells to maintain their normal internal pH thereby also adversely affecting amino acid transport. The effectiveness of ASC as a wash against *Listeria monocytogenes* inoculated on salmon fillets significantly reduced counts (Su and Morrissey, 2003).

This study evaluated the effectiveness of ASC on a RTE roast beef product against *L. monocytogenes* ½ a. The ASC was activated with citric acid at levels of 250, 500, 750, and 1000 ppm.

4.2 MATERIAL AND METHODS

4.2.1 Culture Growth Conditions

*Listeria monocytogenes* strain V7 (serotype ½ a) obtained from the Centers for Disease Control(CDC), Atlanta, GA., U.S.A., that was originally isolated from the blood of an infected individual, was used during this study. The *Listeria monocytogenes* culture was grown for 18 hours in Brain Heart Infusion Broth (BHI) (Difco, Detroit,
Mich., U.S.A.) at 37°C. The pure cultures were stored at -70°C and subcultured twice in BHI Broth at 37°C for 18 hours before being used.

4.2.2 Preparation of Acidified Sodium Chlorite

Sodium chlorite solution (Keeper Professional® Bio-Cide International, Inc.) was mixed with citric acid and sterile distilled water to form the acidified sodium chlorite solution. The mixing process was done according to manufacturer’s instructions with sterile distilled water. The sodium chlorite concentrate (Keeper Professional) was mixed with citric acid and allowed to activate for 10 minutes. The solution concentrate (pH 2.62) was then diluted with sterile distilled water to 250, 500, 750 and 1000 parts per million (ppm). The various treatments were placed in sterile specimen cups and held for one hour at room temperature before being used.

4.2.3 Cooked Roast Beef Samples Dipped into Acidified Sodium Chlorite

Two types of roast beef were used. The samples were obtained from a local meat processor. One roast beef was considered a traditional or regular version of a typical roast beef formula and the other roast beef was of a spicy variety. The spicy roast beef formula contained numerous varieties of peppers, salts, garlic and onion. Both types of roast beef were cut into 5 gram cubes for inoculation studies.

An 18 hour Listeria monocytogenes culture was decimally diluted to 10⁻⁷ in phosphate buffer saline (PBS) solution. One milliliter of the Listeria monocytogenes culture was inoculated onto the surface of the cubed roast beef samples. The cultures were allowed to air dry on the cube roast beef samples for 1 hour under a laminar flow hood. The samples (except control) were then dipped into the different concentrations of ASC treatments for 30 seconds. Control samples received no ASC treatment. The cubed
roast beef samples were allowed to air dry for 20 minutes under the laminar flow hood before placing into either sterile Whirl-Pack® bags (non-vacuumed) or Food Safer7 (Tilia International Inc., San Francisco, CA) vacuum bags with the proper labels. The air was removed from the samples placed into the Food Safer7 vacuum bags and sealed using a Food Safer7 home system. The samples were refrigerated at 4°C and bacterial counts determined at day 0, 7, 14, 21 and 28 by adding 5 ml of PBS to each bag, stomaching for 2 min, making serial dilutions and plating the dilutions onto modified Oxford agar with a selective supplement (cycloheximide, colistin sulphate, Acriflavine, cefoyetan and fosfomycin, each at 20 mg/L) (Oxoid, Hampshire, England). The plates were incubated at 37°C for 48 hours and colony counts expressed in log CFU/g. The only bacteria present on the media were the added cells of *Listeria monocytogenes* strain V7. This was confirmed by plating the control non-inoculated cubed beef samples to ensure that no other Listeria species was present.

### 4.2.4 Cooked Roast Beef Samples Sprayed with Acidified Sodium Chlorite

Regular roast beef samples containing 1.815% lactate and 0.165% diacetate were used during this study. Lactate in combination with diacetate is commonly used in cooked meat and poultry products as a flavoring enhancer or antimicrobial agents. USDA, FSIS permits the use of lactate in combination with diacetate at specified levels to help suppress the growth of low levels of incidental contamination from product spoilage bacteria or pathogens such as *Listeria monocytogenes*. The amount of lactate and diacetate in the roast beef used in this study is within the limits allowed by USDA, FSIS as a flavor enhancer. The amount of lactate and diacetate in the roast beef used in this study should have very little, if any, effect on the growth of high level of inoculation.
(7 logs CFU/g) of *Listeria monocytogenes* used in this study based on in house plant studies done by the local processor.

To simulate plant conditions, the processed cooked beef roasts were cut into 5” x 5” cubes 1” thick (1/16 of the roast beef samples used in the processing plant) (45 g) then each side of each piece was sprayed to cover the product surface. Each piece was sprayed with the different concentrations of ASC solution. The spraying was done at 40 psi using a compressed air operated system with a series J. Dan Mar Company (Huron, OH) spray head.

An 18 hour *Listeria monocytogenes* culture was decimally diluted to $10^7$ in PBS buffer. One milliliter of the *Listeria monocytogenes* culture was inoculated onto the cubed roast beef samples. The cultures were allowed to air dry on the cube roast beef samples for 1 hour under a laminar flow hood. The samples (except for the control) were then sprayed on each side with the different concentrations of ASC treatments. Control samples (0-ppm) received no ASC solution. The cubed roast beef samples were allowed to air dry for 20 minutes under the laminar flow hood before placing into either sterile Whirl-Pack® bags (non-vacuumed) or Food Safer7 (Tilia International Inc., San Francisco, CA) vacuum bags. The air was removed from the samples placed into the Food Safer7 vacuum bags and sealed using a Food Safer7 home system. The samples were refrigerated at 4°C and bacterial counts determined at day 0, 7, 14, 21 and 28 by adding 45 ml of PBS into each bag, stomaching for 2 min, making serial dilutions and plating the dilutions onto Oxford agar with a selective supplement (cycloheximide, colistin sulphate, Acriflavine, cefoyetan and fosfomycin, each at 20 mg/L) (Oxoid, Hampshire, England). The plates were incubated at 37°C for 48 hours and colony counts
expressed in log CFU/g. The only bacteria present on the media were the added cells of *Listeria monocytogenes* strain V7. This was confirmed by plating a control non-inoculated cubed beef samples that had no bacterial growth.

### 4.2.5 Cooked Turkey, Ham, and Hog Head Cheese Samples Sprayed with Acidified Sodium Chlorite

Turkey, ham, and hog head cheese samples containing 1.815% lactate and 0.165% diacetate were used during this study. Lactate in combination with diacetate is commonly used in cooked meat and poultry products as a flavoring enhancer or antimicrobial agents. USDA, FSIS permits the use of lactate in combination with diacetate at specified levels to help suppress the growth of low levels of incidental contamination from product spoilage bacteria or pathogens such as *Listeria monocytogenes*. The amount of lactate and diacetate used in these ready-to-eat meat products is within the limits allowed by USDA, FSIS as a flavor enhancer. The amount of lactate and diacetate in these ready-to-eat meats used during this study should have very little, if any, effect on the growth of high level inoculation of *Listeria monocytogenes* (6 logs CFU/g).

To simulate plant conditions, the processed cooked turkey, ham, and hog head cheese were cut into 5” x 5” cubes 1” thick (1/16 of the ready-to-eat meat samples used in the processing plant) (45 g) then each side of each piece was sprayed with ASC to cover the product surface. Each piece was sprayed with the different concentrations of ASC solution. The spraying was done at 40 psi using a compressed air operated system with a series J. Dan Mar Company (Huron, OH) spray head.

An 18 hour *Listeria monocytogenes* culture was decimally diluted to $10^6$ in PBS buffer. One milliliter of the *Listeria monocytogenes* culture was inoculated onto the
cubed turkey, ham, and hog head cheese samples. The cultures were allowed to air dry on the cube cooked meat samples for 1 hour under a laminar flow hood. The samples (except for the control) were then sprayed on each side with the different concentrations of ASC treatments. Control samples (0-ppm) received no ASC solution. After spraying with ASC the cubed cooked meat samples were placed into Food Safer7 (Tilia International Inc., San Francisco, CA) vacuum bags. The air was removed from the samples placed into the Food Safer7 vacuum bags and sealed using a Food Safer7 home system. The samples were refrigerated at 4°C and bacterial counts determined at day 0, 14, 21 and 28 by adding 45 ml of PBS into each bag, stomaching for 2 min, making serial dilutions and plating the dilutions onto Oxford agar with a selective supplement (cycloheximide, colistin sulphate, acriflavine, cefoyetan and fosfomycin, each at 20 mg/L) (Oxoid, Hampshire, England). The plates were incubated at 37°C for 48 hours and colony counts expressed in log CFU/g. The only bacteria present on the media were the added cells of *Listeria monocytogenes* strain V7. This was confirmed by plating a control non-inoculated cubed turkey, ham and hog head cheese sample that had no bacterial growth.

4.2.6 Color Analysis of Acidified Sodium Chlorite Treated Whole Roast Beef Halves

Whole roast beef halves of the regular and spicy were processed and treated at the meat processing plant. The whole roast beef halves were sprayed with 0, 500, 750 or 1000 ppm of ASC. The treatment solution was sprayed over the entire surface of the meat and then the roast beef halves were vacuum package using a Cryovac (Old Rivers model 8610T-14E, Japan) with clear bags (size 14x20 inches). After the necessary treatments were applied, the meat samples were transported to the Food Safety/Food
Microbiology lab located in the Louisiana Agricultural Chemistry Building on the Louisiana State University campus in a refrigerated truck and stored at 4°C in a walk in cooler until analysis.

The color of the roast beef halves was evaluated on day 0, 7, 14, and 28. The color of the non-treated and ASC treated roast beef samples was measured by a Minolta Spectrophotometer (CM-500d Series, Japan). The spectrophotometer was set to take 5 color readings and average the numbers. The results were given as L*, a*, and b* values. Color readings were taken on four uncut surface areas, the cut surface and two internal surfaces of the roast.

4.2.7 Sensory Analysis

The various uncontaminated roast beef samples (n= 6) were evaluated on taste properties on day 28 of the study. The participants in the sensory evaluation were persons that had over 5 five years experience with the products, but no consumer panel training in sensory evaluation. The treated roast beef samples were also evaluated with roast beef samples pulled from the current day’s line. The participants were asked to evaluate the product on a 9-point hedonic scale (Figure 4.1). A score of one was dislike extremely while a score of 9 was like extremely. Participants were to evaluate overall appearance, texture, taste, odor, overall liking and acceptability. The products sampled were the treated spicy 250 ppm, spicy 500 ppm, spicy control, regular 250 ppm, regular 500 ppm, regular control and a regular product pulled off the line that day.
Figure 4.1: Sensory evaluation form used by untrained sensory panel
4.2.8 Statistical Analysis

The inhibitory effects of the ASC treatments against *Listeria monocytogenes* strain V7 on the surface of the cubed cooked roast beef at 4°C were analyzed by statistical comparisons of all pairs using one-way analysis of the variance (ANOVA) (JUMPIn version 4.0.3, SAS Institute Inc., Cary, N.C., U.S.A.). Statistical significance occurs for P<0.05. All experiments were repeated 3 times with 2 replications per experiment.

4.3 RESULTS AND DISCUSSION

*Listeria monocytogenes* is a major concern to the meat processing industry because it can grow on ready-to-eat meat products at refrigerator temperatures. On October 2, 2003 the USDA FSIS issued a new directive for the control of *Listeria monocytogenes* on ready-to-eat products. Acidified sodium chlorite, which is a combination of any generally recognized as safe (GRAS) acid and sodium chlorite in an aqueous solution, is approved as a direct food additive by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA) to be used for the decontamination of poultry and red-meat carcasses (Anonymous. 1998). In order to help ready-to-eat meat processors meet this new requirement, we investigated acidified sodium chlorite as a possible method for the control of *Listeria monocytogenes* on ready-to-eat roast beef. The sodium chlorite solution was mixed with citric acid because the performance of ASC solution activated with citric acid was better than an ASC solution activated with phosphoric acid (PASC) (Castillo et al., 1999).

Table 4.1 shows various pH ranges for the ASC solution after achieving the desired ASC concentration levels in parts per million (ppm). From the ASC cure at a
Table 4.1: The pH levels of the acidified sodium chlorite solution used as a dipping or spraying antimicrobial agent.

<table>
<thead>
<tr>
<th>ASC CONCENTRATION</th>
<th>PH VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CURE SOLUTION</td>
<td>2.53</td>
</tr>
<tr>
<td>250 PPM</td>
<td>3.25</td>
</tr>
<tr>
<td>500 PPM</td>
<td>3.22</td>
</tr>
<tr>
<td>750 PPM</td>
<td>3.18</td>
</tr>
<tr>
<td>1000 PPM</td>
<td>3.15</td>
</tr>
</tbody>
</table>
level of 1000 ppm, the pH range was 2.53 to 3.15. Similar results were expressed by Su and Morrissey (2003) when the ASC was used on salmon fillets. Their concentration levels of ACS ranged in 50 ppm to 400 ppm with a pH range of 2.89 to 3.29. These pH levels are also in accordance with the United States Department of Agricultures and the Food and Drug Administration guidelines that the sodium chlorite concentrations of 500 to 1,200 ppm in combination with any GRAS acid be at levels to achieve a pH of 2.3 to 3.2 in solution for use on red meat, poultry, seafood, and fruit and vegetables (Lake, 2001; Hwang and Beuchat, 1995).

Table 4.2 shows the effect of different concentrations of acidified sodium chlorite solution as a dip solution had on the growth of *Listeria monocytogenes* inoculated onto the surface of spicy and regular roast beef samples. *Listeria monocytogenes* was able to grow on the surface of both types of control non-treated cooked roast beef samples. We found that at an initial inoculation level of 6.40 CFU/g, *Listeria monocytogenes* grew to 10.50 log CFU/g on the spicy cooked roast beef and 12.0 log CFU/g on the regular roast beef. From day 14 through 28 the *Listeria monocytogenes* counts on the control non-treated spicy roast beef were significantly lower than the control non-treated regular roast beef bacterial counts. On day 14 the non treated spicy roast beef had 0.93 log CFU/g counts lower than then non-treated regular roast beef. By day 28 the non-treated spicy roast beef had greater than a 1 log CFU/g reduction in comparison to the non-treated regular roast beef.

Table 4.3 shows the log reduction of *Listeria monocytogenes* in comparison to the control in that group. The largest difference occurred at day 21 with a 3.38 log CFU/g reduction of *Listeria monocytogenes* counts on the control non-treated spicy roast beef
Table 4.2: Recovery of *Listeria monocytogenes* ≥ a at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of regular and spicy ready-to-eat roast beef after dipping.

<table>
<thead>
<tr>
<th>ASC(^b)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular(^c)</td>
<td>0 ppm</td>
<td>6.42 ±0.03 A</td>
<td>7.25 ±0.05 A</td>
<td>9.48 ±0.50 A</td>
<td>10.93 ±0.12 A</td>
</tr>
<tr>
<td></td>
<td>250 ppm</td>
<td>4.81 ±0.10 C</td>
<td>4.24 ±0.06 B</td>
<td>5.45 ±0.25 C</td>
<td>7.47 ±0.06 B</td>
</tr>
<tr>
<td></td>
<td>500 ppm</td>
<td>4.07 ±0.05 D</td>
<td>4.53 ±0.27 B</td>
<td>5.49 ±0.31 C</td>
<td>7.48 ±0.15 B</td>
</tr>
<tr>
<td></td>
<td>750 ppm</td>
<td>4.45 ±0.04 E</td>
<td>3.94 ±0.06 D</td>
<td>5.39 ±0.35 C</td>
<td>7.53 ±0.13 B</td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
<td>3.35 ±0.11 G</td>
<td>2.51 ±0.07 G</td>
<td>5.42 ±0.34 C</td>
<td>6.07 ±0.01 C</td>
</tr>
<tr>
<td>Spicy(^c)</td>
<td>0 ppm</td>
<td>6.90 ±0.03 A</td>
<td>7.1 ±0.17 A</td>
<td>8.55 ±0.01 B</td>
<td>7.55 ±0.09 B</td>
</tr>
<tr>
<td></td>
<td>250 ppm</td>
<td>5.46 ±0.31 B</td>
<td>4.18 ±0.04 C</td>
<td>5.52 ±0.42 C</td>
<td>6.42 ±0.45 C</td>
</tr>
<tr>
<td></td>
<td>500 ppm</td>
<td>3.93 ±0.05 E</td>
<td>3.34 ±0.01 E</td>
<td>4.44 ±0.01 D</td>
<td>6.03 ±0.31 C</td>
</tr>
<tr>
<td></td>
<td>750 ppm</td>
<td>3.97 ±0.04 C</td>
<td>3.30 ±0.03 E</td>
<td>4.03 ±0.05 D</td>
<td>5.08 ±0.04 D</td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
<td>4.19 ±0.15 E</td>
<td>2.74 ±0.06 F</td>
<td>3.48 ±0.03 E</td>
<td>3.70 ±0.10 E</td>
</tr>
</tbody>
</table>

\(^a\) All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (\(P\geq 0.05\)) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

\(^b\) Different concentrations of sodium chlorite acidified solutions (ASC) for dipping used to treat *L. monocytogenes* contaminated cooked roast beef samples.

\(^c\) Two types of cooked roast beef were used during this study a regular or a spicy formulation.
Table 4.3: Log reductions from Table 4.2 of *Listeria monocytogenes* ½ a at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of regular and spicy ready-to-eat roast beef after dipping.

<table>
<thead>
<tr>
<th>ASCb</th>
<th>Regularc 0 ppm</th>
<th>250 ppm</th>
<th>500 ppm</th>
<th>750 ppm</th>
<th>1000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log Reductions CFU/ga</td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 28</td>
</tr>
<tr>
<td>0 ppm</td>
<td>6.42 ±0.03 A</td>
<td>7.25 ±0.05 A</td>
<td>9.48 ±0.50 A</td>
<td>10.93 ±0.12 A</td>
<td>11.86 ±0.76 A</td>
</tr>
<tr>
<td>250 ppm</td>
<td>1.61C</td>
<td>3.01B</td>
<td>4.03C</td>
<td>3.46B</td>
<td>2.31C</td>
</tr>
<tr>
<td>500 ppm</td>
<td>2.35D</td>
<td>2.72B</td>
<td>3.99C</td>
<td>3.45B</td>
<td>2.43C</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.97E</td>
<td>3.31D</td>
<td>4.09C</td>
<td>3.40B</td>
<td>2.47C</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>3.07G</td>
<td>4.74G</td>
<td>4.06C</td>
<td>4.86C</td>
<td>2.70C</td>
</tr>
</tbody>
</table>

| Spicyc 0 ppm          | 6.90 ±0.03 A   | 7.1 ±0.17 A | 8.55 ±0.01 B | 7.55 ±0.09 B | 10.57 ±0.40 B |
| 250 ppm               | 1.44B          | 2.92C   | 3.03C   | 1.13C   | 1.24C    |
| 500 ppm               | 2.97E          | 3.76E   | 4.11D   | 1.52C   | 2.76D    |
| 750 ppm               | 2.93C          | 3.80E   | 4.52D   | 2.47D   | 4.25E    |
| 1000 ppm              | 2.71E          | 4.36F   | 5.07E   | 3.85E   | 5.85F    |

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P≥0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of sodium chlorite acidified solutions (ASC) for dipping used to treat *L. monocytogenes* contaminated cooked roast beef samples.

c Two types of cooked roast beef were used during this study: a regular or a spicy formulation.
compared to the regular roast beef. On a whole, the spicy roast beef formula reduced the bacterial counts better than the regular roast beef formula. Previous research by other labs have illustrated that spices have the abilities to reduce microbial counts (Billing et al., 1998; Valero et al., 2003)

By day 7 there was no significant differences between the \textit{Listeria monocytogenes} inoculated non-treated controls whereas the ASC treated cooked roast beef samples had greater than 2.70 log CFU/g reductions with the 1000 ppm having the greatest reduction of over 4.00 log CFU/g (Table 4.3). On day 14, there were no significant differences between the different concentrations of ASC treated regular cooked roast beef samples with each concentration having about a 4.00 log CFU/g reduction compared to the control non-treated regular roast beef samples.

Most notable on day 28 was the differences between the ASC treated spicy and regular roast beef samples. The 750 and 1000 ppm ASC treated spicy roast beef samples had 4.25 and 5.85 log CFU/g reductions respectively. The same concentrations of ASC, 750 and 1000 ppm) treated regular roast beef samples had a 2.47 and 2.70 log CFU/g reductions in \textit{Listeria monocytogenes} counts compared to the controls. The ASC solution worked 1.78 log CFU/g or greater in the reduction of Listeria monocytogenes for the spicy roast beef formula.

Immediately following the initial inoculation, all the cooked roast beef samples treated with ASC showed significantly decreased bacterial counts compared to control non-treated samples due to the rapid antimicrobial action of ASC against \textit{Listeria monocytogenes} (Table 4.2 and Table 4.4). The rapid reduction in bacterial counts caused by ASC has also been shown on fresh beef stored at 4°C (Castillo et al., 1999; Lim et al.
Table 4.4: Recovery of *Listeria monocytogenes* ½ a at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of regular ready-to-eat roast beef after spraying then stored under vacuum and non-vacuum packaging conditions.

<table>
<thead>
<tr>
<th>ASCb</th>
<th>Log CFU/ga</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum</td>
<td>0 ppm</td>
<td>7.14 ±0.56 A</td>
<td>7.43 ±0.04 A</td>
<td>9.86 ±0.02 A</td>
<td>11.36 ±0.57 A</td>
<td>12.14 ±0.60 A</td>
</tr>
<tr>
<td></td>
<td>500 ppm</td>
<td>5.29 ±0.06 B</td>
<td>5.59 ±0.34 B</td>
<td>6.62 ±0.33 B</td>
<td>8.46 ±0.09 B</td>
<td>10.64 ±0.04 B</td>
</tr>
<tr>
<td></td>
<td>750 ppm</td>
<td>5.23 ±0.07 B</td>
<td>4.95 ±0.68 BC</td>
<td>6.44 ±0.26 B</td>
<td>8.26 ±0.48 B</td>
<td>10.40 ±0.02 B</td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
<td>4.93 ±0.21 BC</td>
<td>4.26 ±0.46 C</td>
<td>6.34 ±0.34 B</td>
<td>7.46 ±0.10 C</td>
<td>10.29 ±0.26 B</td>
</tr>
<tr>
<td>Non-Vacuumc</td>
<td>0 ppm</td>
<td>7.08 ±0.55 A</td>
<td>7.26 ±0.05 A</td>
<td>9.73 ±0.03 A</td>
<td>11.18 ±0.59 A</td>
<td>12.14 ±0.60 A</td>
</tr>
<tr>
<td></td>
<td>500 ppm</td>
<td>5.15 ±0.07 B</td>
<td>5.39 ±0.19 BC</td>
<td>6.50 ±0.30 B</td>
<td>8.44 ±0.11 B</td>
<td>10.46 ±0.07 B</td>
</tr>
<tr>
<td></td>
<td>750 ppm</td>
<td>5.47 ±0.07 B</td>
<td>4.79 ±0.76 BC</td>
<td>6.41 ±0.34 B</td>
<td>8.31 ±0.44 B</td>
<td>10.33 ±0.05 B</td>
</tr>
<tr>
<td></td>
<td>1000 ppm</td>
<td>4.53 ±0.23 C</td>
<td>4.04 ±0.50 C</td>
<td>6.40 ±0.35 B</td>
<td>7.15 ±0.10 C</td>
<td>10.25 ±0.16 B</td>
</tr>
</tbody>
</table>

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of sodium chlorite acidified solutions (ASC) for spraying used to treat *L. monocytogenes* contaminated cooked roast beef samples.

c The cooked roast beef samples were treated with (vacuum) or with out a vacuum (non-vacuum).
2004). However, an ASC at a low concentration (0.16%) had little effect on the numbers of aerobes, coliforms or *Escherichia coli* on chilled beef carcasses (Gill et al., 2004). Table 4.4 shows the effect on the growth of *Listeria monocytogenes* inoculated onto regular roast beef samples sprayed with different concentrations of ASC then stored at 4°C with or without a vacuum. Roast beef samples sprayed with ASC at day 0 the log CFU/g reductions in counts ranged from 1.85 to 2.21 for vacuumed packaged RTE roast beef compared to control non-treated samples due to the rapid antimicrobial action of ASC against *Listeria monocytogenes* (Table 4.5). By day 7 there were no significant differences between the *Listeria monocytogenes* inoculated non-treated controls whereas the ASC treated cooked roast beef vacuumed samples had 1.84 to 3.1 log CFU/g reductions, with the 1000-ppm having the greatest reduction.

In addition, days 14 and 21 showed the greatest reduction for vacuumed and non-vacuumed packaged RTE roast beef. For vacuum packaged roast beef on day 14 the *Listeria monocytogenes* counts were significantly reduced by 3.24 log CFU/g with samples treated at 500 ppm ASC and 3.52 log CFU/g for samples treated at 1000 ppm when compared to controls (4.5). While on day 21 the reduction in counts ranged from 2.90 log CFU/g for vacuum packaged RTE roast beef treated with 500 ppm ASC and 3.90 log CFU/g for vacuum packaged RTE roast beef treated with 1000 ppm when compared to controls. On day 28, there were no significant differences between the different concentrations of ASC treated regular cooked roast beef samples with each concentration having an inhibitory effect of about 1.5 to 1.89 log CFU/g reduction for vacuum and non-vacuumed roast beef samples compared to the control non-treated regular roast beef samples. During this study, our results showed no significance
Table 4.5: Log reductions from Table 4.4 of *Listeria monocytogenes* ½ a at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of regular ready-to-eat cooked roast beef after spraying then stored under vacuum and non-vacuum packaging conditions.

<table>
<thead>
<tr>
<th>ASCb</th>
<th>Vacuum</th>
<th>Log Reduction CFU/ga</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 7</td>
<td>Day 14</td>
<td>Day 21</td>
<td>Day 28</td>
</tr>
<tr>
<td>0 ppm</td>
<td></td>
<td>7.14 ±0.56 A</td>
<td>7.43 ±0.04 A</td>
<td>9.86 ±0.02 A</td>
<td>11.36 ±0.57 A</td>
<td>12.14 ±0.60 A</td>
</tr>
<tr>
<td>500 ppm</td>
<td></td>
<td>1.85B</td>
<td>1.84B</td>
<td>3.24B</td>
<td>2.90B</td>
<td>1.50B</td>
</tr>
<tr>
<td>750 ppm</td>
<td></td>
<td>1.91B</td>
<td>2.48BC</td>
<td>3.42B</td>
<td>3.10B</td>
<td>1.74B</td>
</tr>
<tr>
<td>1000 ppm</td>
<td></td>
<td>2.21BC</td>
<td>3.17C</td>
<td>3.52B</td>
<td>3.90C</td>
<td>1.85B</td>
</tr>
<tr>
<td>Non-Vacuumc</td>
<td>0 ppm</td>
<td>7.08 ±0.55 A</td>
<td>7.26 ±0.05 A</td>
<td>9.73 ±0.03 A</td>
<td>11.18 ±0.59 A</td>
<td>12.14 ±0.60 A</td>
</tr>
<tr>
<td>500 ppm</td>
<td></td>
<td>1.93B</td>
<td>1.87BC</td>
<td>3.23B</td>
<td>2.74C</td>
<td>1.68B</td>
</tr>
<tr>
<td>750 ppm</td>
<td></td>
<td>1.61B</td>
<td>2.47BC</td>
<td>3.22B</td>
<td>2.87C</td>
<td>1.81B</td>
</tr>
<tr>
<td>1000 ppm</td>
<td></td>
<td>2.55C</td>
<td>3.22C</td>
<td>3.33B</td>
<td>4.03C</td>
<td>1.89B</td>
</tr>
</tbody>
</table>

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P>0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of sodium chlorite acidified solutions (ASC) for spraying used to treat *L. monocytogenes* contaminated cooked roast beef samples.

c The cooked roast beef samples were treated with (vacuum) or without a vacuum (non-vacuum).
Table 4.6: Log reductions of *Listeria monocytogenes* \(^{1/2}a\) on the surface of ready-to-eat roast beef either sprayed or dipped with different concentration of acidified sodium chlorite then stored at 4°C for 28 days.

<table>
<thead>
<tr>
<th>ASC(^b)</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sprayed(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ppm</td>
<td>1.93 ± 0.51CD</td>
<td>1.87 ± 0.24D</td>
<td>3.23 ± 0.78A</td>
<td>2.74 ± 0.49C</td>
<td>1.68 ± 0.54A</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.61 ± 0.49D</td>
<td>2.48 ± 0.71CD</td>
<td>3.33 ± 0.72A</td>
<td>3.07 ± 1.02C</td>
<td>1.81 ± 0.56A</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>2.54 ± 0.41AB</td>
<td>3.23 ± 0.45B</td>
<td>3.33 ± 0.38A</td>
<td>4.03 ± 0.58AB</td>
<td>1.89 ± 0.51A</td>
</tr>
<tr>
<td>Dipped(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>500 ppm</td>
<td>2.37 ± 0.07BC</td>
<td>2.72 ± 0.31BC</td>
<td>3.99 ± 0.19A</td>
<td>3.45 ± 0.19BC</td>
<td>2.43 ± 0.78A</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.98 ± 0.05BCD</td>
<td>3.31 ± 0.08B</td>
<td>4.10 ± 0.36A</td>
<td>3.40 ± 0.17BC</td>
<td>2.47 ± 0.70A</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>3.07 ± 0.11A</td>
<td>4.74 ± 0.06A</td>
<td>4.07 ± 0.38A</td>
<td>4.86 ± 0.13A</td>
<td>2.70 ± 0.76A</td>
</tr>
</tbody>
</table>

\(^a\) All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

\(^b\) Different concentrations of sodium chlorite acidified solutions (ASC) used to treat *L. monocytogenes* contaminated cooked roast beef samples.

\(^c\) *L. monocytogenes* contaminated roast beef samples were either sprayed with ASC or dipped into ASC.
difference between the bacterial reductions (log CFU/g) for vacuumed or non-vacuumed packaged roast beef (Table 4.5).

Furthermore, comparisons between spraying and dipping ASC ready-to-eat roast beef samples (Table 4.6) showed that the *Listeria monocytogenes* counts only at day 7 for the same concentrations of ASC were significantly different from each other. Kemp et al. (2001) showed that the use of a spray wash system for the inside and outside of poultry carcasses to remove visible contamination reduced microbial levels in commercially processed poultry. Our results show that the microbial load can be reduced by dipping or spraying the RTE roast beef.

Table 4.7 & 4.8 illustrates a 28 day study in the reductions of *Listeria monocytogenes* on vacuumed packaged cooked turkey, ham and hog head cheese samples that were spray treated with various concentrations of ASC. *Listeria monocytogenes* was able to grow on the surface of all three non-inoculated ready-to-eat meat products. We found that at an initial inoculation level of about 6 log CFU/g, *Listeria monocytogenes* grew to greater than 9.39 log CFU/g on all three ready-to-eat meat products.

Immediately following the initial inoculation all the cooked vacuumed packaged ready-to-eat meat samples treated with ASC showed significantly decreased bacterial counts compared to control non-treated samples due to the rapid antimicrobial action of ASC against *Listeria monocytogenes* (Table 4.7 & 4.8). By day 14 the ASC treated vacuumed packaged ready-to-eat turkey samples had about 2.33 log CFU/g reductions at 1000 ppm ASC in bacterial counts compared to the control (Table 4.7 & 4.8). Both the ham and hog head cheese inoculated samples had 1.50 log CFU/g reductions at 1000 ppm ACS of *Listeria monocytogenes* compared to the controls at day 14 (Table 4.7 & 4.8).
Table 4.7: Recovery of *Listeria monocytogenes* ½ a at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of vacuumed packaged turkey, ham and hog head cheese.

<table>
<thead>
<tr>
<th>ASCb</th>
<th>a. Turkey</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>6.65 ±0.49 A</td>
<td>8.84 ±0.82 A</td>
<td>9.56 ±0.24 A</td>
<td>10.45 ±0.32 A</td>
<td></td>
</tr>
<tr>
<td>500 ppm</td>
<td>5.25 ±0.68 B</td>
<td>7.14 ±1.14 B</td>
<td>7.79 ±0.07 B</td>
<td>8.75 ±0.26 B</td>
<td></td>
</tr>
<tr>
<td>750 ppm</td>
<td>5.04 ±0.04 B</td>
<td>6.94 ±0.04 B</td>
<td>7.63 ±0.13 B</td>
<td>8.63 ±0.14 B</td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>5.11 ±0.11 B</td>
<td>6.51 ±0.07 B</td>
<td>7.29 ±0.04 B</td>
<td>8.20 ±0.67 B</td>
<td></td>
</tr>
<tr>
<td>b. Ham</td>
<td>0 ppm</td>
<td>6.15 ±0.06 A</td>
<td>8.58 ±0.15 A</td>
<td>8.29 ±0.15 A</td>
<td>9.39 ±0.02 A</td>
</tr>
<tr>
<td>500 ppm</td>
<td>4.94 ±0.19 B</td>
<td>7.31 ±0.39 B</td>
<td>6.46 ±0.51 B</td>
<td>7.52 ±0.02 B</td>
<td></td>
</tr>
<tr>
<td>750 ppm</td>
<td>4.81 ±0.11 B</td>
<td>7.05 ±0.14 B</td>
<td>6.40 ±0.20 B</td>
<td>7.28 ±0.03 B</td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>5.09 ±0.04 B</td>
<td>7.09 ±0.15 B</td>
<td>6.46 ±0.51 B</td>
<td>7.47 ±0.09 B</td>
<td></td>
</tr>
<tr>
<td>c. Hog head cheese</td>
<td>0 ppm</td>
<td>5.96 ±0.03 A</td>
<td>9.04 ± 0.67 A</td>
<td>10.13 ±0.23 A</td>
<td>10.05 ±0.08 A</td>
</tr>
<tr>
<td>500 ppm</td>
<td>5.05 ±0.05 B</td>
<td>8.09 ±0.02 B</td>
<td>8.75 ±0.14 B</td>
<td>8.73 ±0.52 B</td>
<td></td>
</tr>
<tr>
<td>750 ppm</td>
<td>4.96 ±0.04 B</td>
<td>7.97 ±0.11 B</td>
<td>8.76 ±0.05 B</td>
<td>8.70 ±0.35 B</td>
<td></td>
</tr>
<tr>
<td>1000 ppm</td>
<td>5.09 ±0.15 B</td>
<td>7.53 ±0.47 B</td>
<td>8.57 ±0.30 B</td>
<td>8.63 ±0.10 B</td>
<td></td>
</tr>
</tbody>
</table>

*a* All analysis were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P>0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

*b* Different concentrations of sodium chlorite acidified solutions (ASC) used to treat *L. monocytogenes* contaminated cooked turkey, ham, and hog head cheese samples.
Table 4.8: Log reductions from Table 7 of *Listeria monocytogenes* ½ at 4°C for 28 days caused by different concentration of sodium chlorite acidified with citric acid on the surface of vacuumed packaged turkey, ham and hog head cheese.

<table>
<thead>
<tr>
<th>ASC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>a. Turkey</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm</td>
<td>6.65 ±0.49A</td>
<td>8.84 ±0.82A</td>
<td>9.56 ±0.24A</td>
<td>10.45 ±0.32A</td>
</tr>
<tr>
<td>500 ppm</td>
<td>1.4B</td>
<td>1.71B</td>
<td>1.77B</td>
<td>1.70B</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.61B</td>
<td>1.90B</td>
<td>1.93B</td>
<td>1.82B</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>1.54B</td>
<td>2.33B</td>
<td>2.27B</td>
<td>2.25B</td>
</tr>
<tr>
<td><strong>b. Ham</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ppm</td>
<td>6.15 ±0.06A</td>
<td>8.58 ±0.15A</td>
<td>8.29 ±0.15A</td>
<td>9.39 ±0.02A</td>
</tr>
<tr>
<td>500 ppm</td>
<td>1.21B</td>
<td>1.27B</td>
<td>1.83B</td>
<td>1.87B</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.34B</td>
<td>1.53B</td>
<td>1.89B</td>
<td>2.11B</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>1.06B</td>
<td>1.49B</td>
<td>1.83B</td>
<td>1.92B</td>
</tr>
<tr>
<td><strong>c. Hog head cheese</strong></td>
<td>0 ppm</td>
<td>5.96 ±0.03A</td>
<td>9.04 ±0.67A</td>
<td>10.13 ±0.23A</td>
</tr>
<tr>
<td>500 ppm</td>
<td>0.91B</td>
<td>0.92B</td>
<td>1.39B</td>
<td>1.32B</td>
</tr>
<tr>
<td>750 ppm</td>
<td>1.0B</td>
<td>1.10B</td>
<td>1.37B</td>
<td>1.35B</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>0.87B</td>
<td>1.51B</td>
<td>1.56B</td>
<td>1.42B</td>
</tr>
</tbody>
</table>

<sup>a</sup> All analysis were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

<sup>b</sup> Different concentrations of sodium chlorite acidified solutions (ASC) used to treat *L. monocytogenes* contaminated cooked turkey, ham, and hog head cheese samples.
On day 28, there were no significant differences in the reduction of *Listeria monocytogenes* counts between the different concentrations of ASC treated ready-to-eat meat samples with the turkey samples having about 2.00 log CFU/g reductions, ham 1.92 log CFU/g reductions and hog head cheese 1.42 log CFU/g reductions as compared to the controls non-treated ready-to-eat meat samples.

The outer surface of the roast beef is covered in a coating that did not change color at all. The exposed cut side of the roast beef demonstrated measurable detectable differences. Table 4.9 evaluates the color of the regular roast beef samples treated with ASC on the exposed cut side of a roast beef half. The analysis of the color values demonstrated that there was a slight variation in the color but the differences were not significant. The similar results were noticed with the spicy roast beef. However, the spicy roast beef started off with higher total redness values because of the spices used in the product formulation. Lim et al. (2004) noticed that loss of redness and light color of fresh beef surfaces coincides with decreases in pH. However, our study with process beef did not observe these color changes with ASC. Color variation of fresh beef samples can occur due to the gender, age or species of animals used (Page et al, 2001). The slight variation in color found in our study is postulated to be due to differences in beef samples before processing.

Figure 4.2 shows the sensory responses for regular and spicy roast beef samples sprayed with 250 ppm or 500 ppm ASC. A hedonic score value of 6 means the product was slightly liked, while a score of 7 and 8 means the product was moderately liked and liked very much, respectively. The treated samples were evaluated 28 days after storage.
Table 4.9: The evaluation of color on the cut side of a ready-to-eat regular roast beef halves treated with acidified sodium chlorite at different concentrations\textsuperscript{a} for 28 days.

<table>
<thead>
<tr>
<th></th>
<th>0 ppm</th>
<th>500 ppm</th>
<th>750 ppm</th>
<th>1000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
<td>a*</td>
<td>b*</td>
<td>L</td>
</tr>
<tr>
<td><strong>DAY 0</strong></td>
<td>42.07</td>
<td>9.07</td>
<td>8.74</td>
<td>42.22</td>
</tr>
<tr>
<td><strong>DAY 14</strong></td>
<td>42.11</td>
<td>9.24</td>
<td>9.28</td>
<td>40.72</td>
</tr>
<tr>
<td><strong>DAY 28</strong></td>
<td>43.70</td>
<td>9.99</td>
<td>11.04</td>
<td>42.47</td>
</tr>
</tbody>
</table>

\textsuperscript{a} All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P > 0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).
Figure 4.2: Sensory panel evaluation of 250 or 500 ppm of sodium chlorite acidified treated cooked regular (R) or spicy (S) cooked roast beef products after 28 days storage at 4°C in comparison to control non-treated (Control) or fresh product pulled off the line the day of the study (Line).
The product pulled off the line that day was liked very much by the panel. In comparing the samples there was an overall 1.5 point reduction in the treated samples scores as compared to the fresh sample pulled from the production line. Still, in total there was very little variation between the different products.

Our results have shown that ASC is effective in inhibiting the growth of *Listeria monocytogenes* when this pathogen was grown on the surface of ready-to-eat roast beef at refrigerator temperatures. Furthermore, ASC had no effect on the sensory properties of the non-inoculated cooked roast beef products. With the recurring recalls of ready-to-eat meat and poultry products due to contamination by *Listeria monocytogenes* there is a clear need to develop additional methods to prevent economic loss and possible deaths that can occur from foodborne listeriosis infections. The method reported here should prove helpful in this quest.

4.4 REFERENCES


CHAPTER 5

THE USE OF EDIBLE CHITOSAN FILM ON READY-TO-EAT MEAT PRODUCTS FOR THE CONTROL OF LISTERIA MONOCYTOGENES
5.1 INTRODUCTION

The heightened demands by consumers for quality and freshness of food products have given rise to the development and implementation of edible films. Edible films or coatings are defined as continuous matrices that can be prepared from proteins, polysaccharides, and lipids (Cagri et al, 2004). In the ready-to-eat foods industry the use of edible films help to maintain product quality, enhance sensory properties, improve product safety, and increase the shelf life of products. Our study investigated chitosan as a coating to control Listeria monocytogenes on the surface of ready-to-eat roast beef.

Chitosan is derived by the deacetylation of chitin (poly-β-(1→4) N-acetyl-D-glucosamine. It is a major component of the shells of crustaceans such as crab, shrimp, and crawfish (No et al., 2002). Chitosan has been reviewed for commercial application in the biomedical, food and chemical industries (Knorr, 1984; Muzzarell, 1977). Due to its biological activities, such as the antimicrobial activity (Tsai et al., 2004; No et al., 2002; Tsai et al., 1999; Chen et al., 1998; Fang et al., 1994; Sekiguchi et al., 1994; Sudarshan et al., 1992; Kendra and Hadwiger, 1984;), antitumor (Tokoro et al., 1988; Suzuki et al., 1986), and hypocholesterolemic functions (Sugano et al., 1992), chitosan and the chitosan oligomers have received considerable attention. Chitosan as an antimicrobial film is a good choice due to its film-forming properties (Darmadji and Izumimoto, 1994). No et al. (2003) found that chitosan generally showed a stronger bactericidal effect against gram-positive bacteria than gram-negative bacteria. In addition, chitosan films have been shown to have antimicrobial activity against mold. A chitosan film dissolved into 1% acetic acid reduced grey mold numbers on the surface of table grapes (Romanazzi et al., 2002).
The antifungal and antimicrobial activities of chitosan are believed to originate from its polycationic nature (Kim et al., 2003 and Roller et al., 2000). The antimicrobial action of chitosan is hypothesized to be mediated by the electrostatic forces between the protonated amino group (NH$_2$) in chitosan and the negative residues at cell surfaces (Tsai et al., 1999). The number of protonated amino groups (NH$_2$) present in chitosan increases with increased degrees of deacetylation (DD) which influences antimicrobial activity (Tsai et al., 2002). Liu et al. (2004) states that the bactericidal activity of chitosan is caused by the electrostatic interaction between NH$_3^+$ groups of chitosan and the phosphoryl groups of the phospholipid components of the cell membrane. The antimicrobial activity is likely to differ based on the preparation methods used to convert chitin to chitosan. This change would then cause differences in the deacetylation and the distribution at acetyl groups, chain length, and the conformational structure of chitin and the chitosan molecule. All these factors will affect the characteristics of chitin and chitosan (Tsai et al 2002; Terbojevich et al., 1992). Furthermore, the antibacterial effect of chitosan and chitosan oligomers are dependent on the molecular weight (Jeon et al., 2001; Liu et al., 2001; Uchida et al., 1989).

5.1.1 Lactic Acid

Lactic acid, 2-hydroxypropionic acid, is a weak organic acid. Lactic acid is formed by the reduction of pyruvic acid in biological systems. Microorganisms such as Lactobacillus, Bacillus, Sporolactobacillus, Lactococcus, Enterococcus, Streptococcus, Pediococcus and Leuconostoc are capable of producing large quantities of lactic acid. In the food industry, lactic acid can be used for the acidification potential, pH regulation
property of sodium and potassium lactates, reduction of water activity, synergism with antioxidants and antimicrobial activity (Bogaert et al., 2000).

5.1.2 Acetic Acid

Acetic acid, ethanoic acid, is a colorless liquid with a pungent odor and a sour taste that is generally listed as vinegar. It is a low cost, generally recognized as safe (GRAS) substance which serves as an excellent solvent for organic compounds. The formation of acetic acid is done by a four step reaction with (1) the conversion of starch to sugar by amylases, (2) the anaerobic conversion of sugars to ethanol by yeast fermentation, (3) the conversion of ethanol to hydrated acetaldehyde, and (4) dehydrogenation to acetic acid by aldehyde dehydrogenase (Marshall et al, 2000). The last two steps are performed aerobically with acetic acid bacteria, such as Acetobacter aceti, Gluconobacter suboxydans (Park et al., 1991; Ebner, 1982) and Clostridium thermoaceticum (Cheryan et al., 1997).

When acetic acid, lactic acid and citric acid and hydrochloric acids have been tested on Listeria monocytogenes, acetic acid reduced the numbers of Listeria monocytogenes more effectively than any other acid (Young and Foegeding, 1993; Ita and Hutkins, 1991). It has also been observed that acetic acid was better that lactic or citric acid in controlling Escherichia coli O157:H7 at a pH of 4.7 at 30°C.

This study evaluates the effectiveness of chitosan dissolve in lactic acid or acetic acid adjusted to a pH of 5.6 at different molecular weights to reduce Listeria monocytogenes on ready-to-eat roast beef.
5.2 MATERIALS AND METHODS

5.2.1 Culture Growth Conditions

Listeria monocytogenes strain (serotype ½ a) obtained from the Centers for Disease Control, Atlanta (CDC), GA., U.S.A., that was originally isolated from the blood of an infected individual, was used during this study. The Listeria monocytogenes culture was grown for 18 hours in Brain Heart Infusion Broth (BHI) (Difco, Detroit, Mich., U.S.A.) at 37°C. The pure cultures were stored at -70°C and subcultured twice in BHI Broth at 37°C for 24 h before being used.

5.2.2 Preparation of Chitosan Solution

Chitosan with molecular weights (Mw) of 470 kDa or 1106 kDa were designated as having a low or high molecular weight, respectively for this study. Chitosan with the Mw of 470 kDa was purchased from Keumho Chemical (Seoul, Korea) while the chitosan with Mw of 1106 kDa was purchased from Premix Ingredients (Avaldsnes, Norway). The low molecular weight chitosan came from shrimp shells, while the high molecular weight chitosan came from crab shell. The chitosan came in a powder form and was stored at freezer temperatures until dissolved. The chitosan was dissolved in 1% lactic or 1% acetic acid individually at 1% concentration on dry basis and diluted to 0.5% concentration with distilled water (No et al., 2002). All solutions were adjusted to a pH of 5.6. Table 5.1 illustrates the eight treatments that were studied. In the study lactic acid and acetic acid were used as control treatments along with no use of culture or chitosan as a control.
Table 5.1. Edible chitosan film treatments applied to ready-to-eat roast beef cubes.

<table>
<thead>
<tr>
<th>Percentage Acid</th>
<th>Molecular Weight $^a$</th>
<th>Acid Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.5</td>
<td>High</td>
</tr>
<tr>
<td>2.</td>
<td>0.5</td>
<td>Low</td>
</tr>
<tr>
<td>3.</td>
<td>1.0</td>
<td>High</td>
</tr>
<tr>
<td>4.</td>
<td>1.0</td>
<td>Low</td>
</tr>
<tr>
<td>5.</td>
<td>0.5</td>
<td>High</td>
</tr>
<tr>
<td>6.</td>
<td>0.5</td>
<td>Low</td>
</tr>
<tr>
<td>7.</td>
<td>1.0</td>
<td>High</td>
</tr>
<tr>
<td>8.</td>
<td>1.0</td>
<td>Low</td>
</tr>
</tbody>
</table>

$^a$ Molecular Weight (Mw) of 470 kDa is designated as low and 1106 kDa is designated as high.
5.2.3 Treatment of Cooked Roast Beef Samples with Chitosan Solution

An overnight *Listeria monocytogenes* culture was decimally diluted to 7.0 logs CFU/g (high inoculations). One milliliter of the *L. monocytogenes* culture was inoculated onto the 5-gram cubed roast beef samples. The cultures were allowed to air dry on the cubed roast beef samples for 1 hour under a laminar flow hood. The samples were then dipped into the different types of chitosan solution for 30 seconds. The cubed roast beef samples were allowed to air dry for one hour under the laminar flow hood before placing into sterile Whirl-Pack® bags with the proper labels.

The samples were refrigerated at 4°C and bacterial counts determined at days 0, 7, 14, 21 and 28 by adding 5 ml of Phosphate Buffer Saline (PBS) to each bag, stomaching for 2 min, making serial dilutions and plating the dilutions onto Oxford agar with a selective supplement (cycloheximide, colistin sulphate, acriflavine, cefoyetan and fosfomycin, each at 20 mg/L) (Oxoid, Hampshire, England). The plates were incubated at 37°C for 48 hours and colony counts expressed in log CFU/g. The only bacteria present on the media were the added cells of *Listeria monocytogenes* strain V7. This was confirmed by plating the control non-inoculated cubed beef samples that had no bacterial growth. This was done to ensure that no other *Listeria* species was present.

5.2.4 Statistical Analysis

The inhibitory effects of the ACS treatments against *Listeria monocytogenes* strain V7 on the surface of the cubed cooked roast beef at 4°C were analyzed by statistical comparisons using statistical significance at a level of p>0.05 using One-way ANOVA (JUMPIn version 4.0.3, SAS Institute Inc., Cary, N.C., U.S.A.). All experiments were repeated 3 times with 2 replications per experiment.
5.3 RESULTS AND DISCUSSION

During the past year several ready-to-eat products have been recalled due to *Listeria monocytogenes* contamination (CDC, 1999). The *Listeria monocytogenes* strain used during this study was able to grow on the surface of the ready-to-eat roast beef product. The initial levels of 6 logs CFU/g grew to over 11 log CFU/g on the control samples. This study along with others done in our lab have shown that *Listeria monocytogenes* cultures can grow to high levels on ready-to-eat roast beef products.

Tables 5.2 and 5.3 show the antimicrobial effects of edible chitosan coating dissolved with lactic acid against *Listeria monocytogenes* on the surface of ready-to-eat roast beef stored at 4°C for 28 days. By day seven, *Listeria monocytogenes* counts were significantly reduced when the ready-to-eat roast beef cubes were coated 0.5% lactic acid with the low molecular weight chitosan compared to the control. There were no significant differences between the chitosan treatments on day 14, but the treatments were significantly different from the control and lactic acid treatments. On the day 14 there was a 2.0 to 2.5 log reduction in *Listeria monocytogenes* counts with all chitosan treatments when compared to the control. However by days 21 and 28 there was no significant difference in *Listeria monocytogenes* counts between lactic acid treated ready-to-eat roast beef and the low molecular weight chitosan dissolved with 1.0 % and 0.5% lactic acid. The *Listeria monocytogenes* counts on the ready-to-eat roast beef were reduced about 1 log CFU/g when treated with lactic acid by days 21 and 28. The most effective chitosan coating was the low molecular weight chitosan that had about 1 log CFU/g reduction at day 28.
Table 5.2: Recovery of *Listeria monocytogenes* ½ at 4°C for 28 days caused by different edible film treatments of chitosan dissolved in lactic acid on the surface of ready-to-eat roast beef.

<table>
<thead>
<tr>
<th>Chitosan Treatment b</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.41± 0.07 A</td>
<td>9.87± 0.04 A</td>
<td>10.29± 0.67 A</td>
<td>11.53± 0.18 A</td>
<td>11.72± 0.21 A</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>6.67± 0.07 A</td>
<td>7.07± 1.06 B</td>
<td>10.07± 0.67 A</td>
<td>10.42± 0.15 C</td>
<td>10.44± 0.15 D</td>
</tr>
<tr>
<td>High 1%</td>
<td>6.03± 0.43 A</td>
<td>7.20± 0.55 B</td>
<td>8.79± 0.55 B</td>
<td>11.10± 0.17 B</td>
<td>11.03± 0.25 B</td>
</tr>
<tr>
<td>Low 1%</td>
<td>6.49± 0.13 A</td>
<td>8.31± 0.43 B</td>
<td>9.03± 0.56 B</td>
<td>10.47± 0.22 B</td>
<td>10.52± 0.17 D</td>
</tr>
<tr>
<td>High 0.5%</td>
<td>6.06± 0.01 A</td>
<td>8.20± 0.47 B</td>
<td>8.89± 0.60 B</td>
<td>11.13± 0.23 C</td>
<td>11.14± 0.32 BC</td>
</tr>
<tr>
<td>Low 0.5%</td>
<td>6.49± 0.01 A</td>
<td>7.20± 0.39 B</td>
<td>8.64± 0.55 B</td>
<td>10.67± 0.11 C</td>
<td>10.70± 0.16 C</td>
</tr>
</tbody>
</table>

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P>0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples.
Table 5.3: Log reductions from Table 5.2 of *Listeria monocytogenes* ½ *a* at 4°C for 28 days caused by different edible film treatments of chitosan dissolved in lactic acid on the surface of ready-to-eat roast beef.

<table>
<thead>
<tr>
<th>Chitosan Treatment</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.41± 0.07 A</td>
<td>9.87± 0.04 A</td>
<td>10.29± 0.67 A</td>
<td>11.53± 0.18 A</td>
<td>11.72± 0.21 A</td>
</tr>
<tr>
<td>Lactic Acid</td>
<td>0.26 A</td>
<td>2.80 B</td>
<td>0.22 A</td>
<td>1.11 C</td>
<td>1.28 D</td>
</tr>
<tr>
<td>High 1%</td>
<td>0.38 A</td>
<td>2.67 B</td>
<td>1.50 B</td>
<td>0.43 B</td>
<td>0.69 B</td>
</tr>
<tr>
<td>Low 1%</td>
<td>0.08 A</td>
<td>1.56 B</td>
<td>1.26 B</td>
<td>1.06 B</td>
<td>1.20 D</td>
</tr>
<tr>
<td>High 0.5%</td>
<td>0.35 A</td>
<td>1.67 B</td>
<td>1.40 B</td>
<td>0.40 C</td>
<td>0.58 BC</td>
</tr>
<tr>
<td>Low 0.5%</td>
<td>0.08 A</td>
<td>2.67 B</td>
<td>1.65 B</td>
<td>0.86 C</td>
<td>1.02 C</td>
</tr>
</tbody>
</table>

*a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P≥ 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

*b Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples.
The effects of the different molecular weight chitosans dissolved with acetic acid at 1.0% and 0.5% are shown in Tables 5.4 and 5.5. On day 0 there were no significant differences between the treatments and the control. On day 7 there was greater than a 2.5 log reduction in *Listeria monocytogenes* counts with the low molecular weight 1% acetic acid chitosan coating in comparison to the control. The other chitosan treatments on day seven had a significant 2 log CFU/g reduction in *Listeria monocytogenes* counts when compared to the non-treated control. The chitosan treatments on day 14 were not significantly different from one another. Day 14 showed a 2.0 to 2.5 log reduction in *Listeria monocytogenes* counts for the 1% acetic acid chitosan treatments when compared to the control. By day 21, the 1% acetic acid chitosan solutions had significantly lower counts than the chitosan dissolved with 0.5% acetic acid. This trend was also seen on day 28 of the study.

Our results have shown that at 1% acetic acid chitosan was more effective in reducing *Listeria monocytogenes* counts than the 0.5% acetic acid chitosan. This was also observed by Romanazzi et al. (2002) in grey mold on table grapes. However the ability of *Listeria monocytogenes* to grow on the surface of the ready-to-eat roast beef regardless of the chitosan treatment was observed. Coma et al. (2002) also observed the ability of *Listeria monocytogenes* to grow regardless of chitosan treatments using cheese. This could be explained because the antimicrobial activity of chitosan films decreases over time because of the decreased availability of amino groups on chitosan (Cargi et al., 2004; Coma et al., 2002).
Table 5.4. Recovery of *Listeria monocytogenes* ½ at 4°C for 28 days caused by different edible film treatments of chitosan dissolved in acetic acid on the surface of ready-to-eat roast beef.

<table>
<thead>
<tr>
<th>Chitosan Treatment b</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.65± 0.45 A</td>
<td>9.11± 1.11 A</td>
<td>10.29±0.67AB</td>
<td>11.53± 0.18 A</td>
<td>11.72± 0.21 A</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>6.34± 0.62 A</td>
<td>7.13± 0.38 B</td>
<td>9.73±0.58AB</td>
<td>10.36± 0.16 B</td>
<td>10.40± 0.11 B</td>
</tr>
<tr>
<td>High 1%</td>
<td>5.90± 0.73 A</td>
<td>7.41± 0.52 B</td>
<td>8.30± 0.70C</td>
<td>8.88± 0.06C</td>
<td>8.93± 0.07 C</td>
</tr>
<tr>
<td>Low 1%</td>
<td>5.91± 0.53 A</td>
<td>5.98± 0.57 B</td>
<td>8.39± 0.70C</td>
<td>8.30± 0.22D</td>
<td>8.41± 0.11 D</td>
</tr>
<tr>
<td>High 0.5%</td>
<td>5.98± 0.76 A</td>
<td>7.03± 0.44 B</td>
<td>8.73± 0.43BC</td>
<td>9.43± 0.12E</td>
<td>9.50± 0.04 E</td>
</tr>
<tr>
<td>Low 0.5%</td>
<td>5.94± 0.65 A</td>
<td>6.95± 0.43 B</td>
<td>8.39± 0.67C</td>
<td>9.38± 0.16D</td>
<td>9.37± 0.17 D</td>
</tr>
</tbody>
</table>

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P> 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples.
Table 5.5. Log reductions from Table 5.4 of *Listeria monocytogenes* ½ a at 4°C for 28 days caused by different edible film treatments of chitosan dissolved in acetic acid on the surface of ready-to-eat roast beef.

<table>
<thead>
<tr>
<th>Chitosan Treatment b</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.65± 0.45 A</td>
<td>9.11± 1.11 A</td>
<td>10.29±0.67AB</td>
<td>11.53± 0.18 A</td>
<td>11.72± 0.21 A</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>0.31 A</td>
<td>1.98 B</td>
<td>0.56 AB</td>
<td>1.17 B</td>
<td>1.32 B</td>
</tr>
<tr>
<td>High 1%</td>
<td>0.75 A</td>
<td>1.70 B</td>
<td>1.99 C</td>
<td>2.65C</td>
<td>2.79 C</td>
</tr>
<tr>
<td>Low 1%</td>
<td>0.74 A</td>
<td>3.13 B</td>
<td>1.90 C</td>
<td>3.23D</td>
<td>3.31 D</td>
</tr>
<tr>
<td>High 0.5%</td>
<td>0.67 A</td>
<td>2.08 B</td>
<td>1.56 BC</td>
<td>2.1 E</td>
<td>2.22 E</td>
</tr>
<tr>
<td>Low 0.5%</td>
<td>0.71A</td>
<td>2.16 B</td>
<td>1.90 C</td>
<td>2.15 D</td>
<td>2.35 D</td>
</tr>
</tbody>
</table>

a All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P>0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

b Different concentrations of chitosan solutions used to treat *L. monocytogenes* inoculated ready-to-eat roast beef samples.
Figure 5.1 shows the effect of the lactic acid and acetic acid solutions used to dissolve chitosan on day 28 in the reduction of the *Listeria monocytogenes* inoculated on the surface of ready-to-eat roast beef. The acetic acid solutions at 0.5% and 1% were the most effective in reducing *Listeria monocytogenes*. Furthermore, the acetic acid solution at 1% was significantly more effective than the acetic acid solution at 0.5%. However, there was no significant difference between the chitosan dissolved in different lactic acid concentrations.

Our study demonstrated that edible chitosan films dissolved in acetic acid or lactic acids could be useful as coatings to control *Listeria monocytogenes* on the surface of ready-to-eat roast beef.

### 5.4 REFERENCES


Figure 5.1. The effect of the lactic acid and acetic acid solutions used to dissolve chitosan on day 28 in the growth of the *Listeria monocytogenes* culture.


CHAPTER 6

ANTIMICROBIAL EFFECT OF CRANBERRY JUICE
AS A MARINATE FOR THE CONTROL OF
LISTERIA MONOCYTOGENES ON THE SURFACE
OF RAW SHRIMP
6.1 INTRODUCTION

In recent years, the United States general public has been concerned with the microbial safety and quality of food products. The average consumer has become concerned with the microbial safety of ready-to-eat meat products because *Listeria monocytogenes* (LM) has caused a number of outbreaks and deaths associated with these products. *Listeria monocytogenes* is a foodborne pathogen that is capable of surviving at refrigerated temperatures. It is due to this fact that it is a problem in ready-to-eat meat products. One possible solution would be the use of natural compounds such as cranberries. Consumer concerns have come about due to foodborne pathogens such as *Listeria monocytogenes*. *Listeria monocytogenes* is a foodborne pathogen that has been linked to outbreaks and sporadic cases of listeriosis (Ryser, 1999). It is estimated by the Centers for Disease and Control that approximately 2,500 cases of listeriosis are reported each year with 500 of the cases resulting in death (Mead et.al, 1999). The incidence of *L. monocytogenes* is relatively low, but the consequences of infection may be severe. An estimated 2 to 6% of the healthy population harbors *L. monocytogenes* in their intestinal tract without signs of illness.

Consumers have demanded that food products have a more natural content rather than chemical content. The use of natural food ingredients could be one way to control and prevent foodborne illnesses and outbreaks. Food additives with dual functions, like phenolic antioxidants and spices, could lead to a reduction in the number of additives used in the food supply. The ability of phenolic compounds to scavenge harmful free radical and inhibit oxidative reactions with vital biological molecules are important factors (Lodovici et al., 2001; Kinsella et al., 1993; Block et al, 1992). Phenolic
compounds are the constituents of many fruits (Murphy et al., 2003; Zuo et al., 2002). It has been shown that phenolic compounds are effective in inhibiting and deterring microbial growth (Thompson et al., 2001). Previous studies have shown fruits such as grape, apple, orange, prune, and berries have high phenolic contents (Zuo et al., 2002; Häkkinen et al., 1999; Macheix et al., 1990). Cranberry (Vaccinium macrocarpon), a native fruit in North American has caught the public’s attention due to its potential health benefits in reducing bacterial adhesion in urinary tract infections (UTI) of Escherichia coli and stomach ulcers (Burger et al., 2000; Foo et al., 2000), anticancer effects and contain significant amounts of antioxidant compounds (Murphy et al., 2003; Wang et al., 2000; Wilson et al., 1998). Cranberries contain the highest content of total phenolics per serving and by weight among 20 fruits analyzed in 2001 (Vinson et al., 2001). Cranberries provide numerous types of phenolic compounds that may aid in hindering or inhibiting microbial growth (Thompson et al., 2001, Macheix et al., 1990).

In addition to the phenolic compounds cranberries have the intrinsic characteristic of a low pH and high titratable acidity (Nogueira et al., 2003). These intrinsic properties create a hostile environment for bacterial growth and survival. The primary acid in cranberries is benzoic acid. Table 6.1 list a number of phenolic and benzoic compounds that have been identified in cranberries. Studies have shown that benzoic acid in combination with plant oils such as fennel or basil has a synergistic property to reduce Listeria monocytogenes count (Fyfe et al., 1998). Our study investigated the antimicrobial effect of cranberry juice against Listeria monocytogenes in vitro and on raw shrimp.
Table 6.1. Identified phenolic and benzoic compounds in cranberries (*Vaccinium macrocarpon*)

<table>
<thead>
<tr>
<th>Phenolic Compound Name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 2,3-Dihydroxybenzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>2. 2,4-Dihydroxybenzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>3. Benzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>4. <em>cis</em>- 3-<em>O</em>-p-Hydroxycinnamoyl Ursolic Acid</td>
<td>Murphy et al., 2003</td>
</tr>
<tr>
<td>5. Catechin (flavan-3-ol)</td>
<td>Prior et al., 2001</td>
</tr>
<tr>
<td>6. Ferulic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>7. <em>m</em>-Hydroxybenzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>8. <em>p</em>-Caumaric Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>9. <em>p</em>-Hydroxybenzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>10. <em>p</em>-Hydroxphenylacetic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>11. Phenylboronic Acid</td>
<td>Murphy et al., 2003</td>
</tr>
<tr>
<td>12. <em>o</em>-Hydroxybenzoic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>13. <em>o</em>- Hydroxycinnamic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>14. <em>o</em>-Phthalic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>15. Sinapic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>16. <em>trans</em>- 3-<em>O</em>-p-Hydroxycinnamoyl Ursolic Acid</td>
<td>Murphy et al., 2003</td>
</tr>
<tr>
<td>17. <em>trans</em>-Cinnamic Acid</td>
<td>Zuo et al., 2002</td>
</tr>
<tr>
<td>18. Vanillie Acid</td>
<td>Zuo et al., 2002</td>
</tr>
</tbody>
</table>
6.2 MATERIALS AND METHODS

6.2.1 Culture Growth Condition

*Listeria monocytogenes* strain V7 (serotype ½ a) obtained from the Centers for Disease Control, Atlanta, GA., U.S.A., a strain originally isolated from blood of an infected individual, was used during this study. The bacterial culture was grown for 18 hours in Brain Heart Infusion (BHI) Broth (Difco, Detroit, Mich., U.S.A.) at 37°C.

6.2.2 Effect of Cranberry Juice Concentration on the Reduction of *Listeria monocytogenes*

The culture was separated into 1ml fractions and centrifuged @14,000 rpm for 10 minutes. The bacterial pellets were resuspended into different 1 ml concentrations of filtered cranberry juice (Ocean Spray® Cranberry Juice Cocktail from Concentrate) bought from a local grocery chain. The cranberry juice contained 27% cranberry juice to volume with the balance of the ingredients as high fructose corn syrup and ascorbic acid. The juice concentration studied: 27%, 24%, 22%, 19%, 16%, 14%, 2.7%, 0.27%, and 0%. The samples were then incubated at 37°C for one hour.

6.2.3 Time Required for Cranberry Juice to Reduce *Listeria monocytogenes*

The samples were analyzed at 30, 60, 90, 120, and 150 minutes intervals. The treatments were decimally diluted with Phosphate Buffer Saline (PBS) solution. The samples were spread plated onto modified Oxford (Difco, Detroit, Mich., U.S.A.) plates and incubated at 37°C for 48 hours.

6.2.4 Effect of Cranberry Juice pH on the Reduction of *Listeria monocytogenes*

The bacterial culture was grown for 16 hours in Brain Heart Infusion Broth (Difco, Detroit, Mich., U.S.A.) at 37°C. The pH of 27% cranberry juice and BHI (Difco, Detroit, Mich., U.S.A.) broth as controls were adjusted to a pH level of 2, 3, 4, 5, 6, 7, 8,
9, 10 or 11 with 1M hydrogen chloride or 1M sodium hydroxide and measured by the pH meter (ThermoOrion, model 210At, Boston, MA). The culture was separated into 1ml fractions and centrifuged @14,000 rpm for 10 minutes. The bacterial pellets were resuspended into different 1 ml concentrations of cranberry juice or BHI broth at the different pH levels. The control sample was resuspended in 1 mL of Phosphate Buffer Saline (PBS) with pH of seven. All samples were placed in a shaking incubator for one hour. The samples were then decimally diluted and spread plated onto modified Oxford agar (Difco, Detroit, Mich., U.S.A.) plates and incubated at 37°C for 48 hours.

6.2.5 **Effect of Cranberry Juice Reduction of *Listeria monocytogenes* in Raw Shrimp**

Raw peel shrimp were purchased from a local grocer. The shrimp were weighed and aseptically adjusted to 5 grams per shrimp. The shrimp were then inoculated with 100µL of an overnight *Listeria monocytogenes* ½ a culture. After inoculation the shrimp were allowed to air dry for 2 minutes under a Class II A/B3 Biological Safety Cabinet (Forma Scientific model 1184, Ohio). The shrimp were then bagged without a vacuum at temperatures of 37°C or 4°C. The samples were then examined at 30 minutes 60 minutes and 24 hours with the addition of phosphate buffer saline (PBS) in the bags. The samples were decimally diluted and spread plated onto modified Oxford (Difco, Detroit, Mich., U.S.A.) plates and incubated at 37°C for 48 hours.

6.2.6 **Statistical Analysis**

The bacterial counts were transformed to log_{10} CFU/ml. The counts were compared for statistical significance at a level of p>0.05 using One-way ANOVA. The data was analyzed by JUMPIn version 4.0.3 (© 1989 - 2000 SAS Institute Inc.)
6.3 RESULTS AND DISCUSSION

The cranberry juice (Ocean Spray® Cranberry Juice Cocktail from Concentrate) was purchased from a local chain grocer. The pH of the cranberry juice straight from the container had an average pH of 2.5 after 6 total readings from 2 different containers (data not shown). The cranberry juice was plated onto Total Plate Count (Difco, Detroit, Mich., U.S.A.) agar and modified Oxford agar (Difco, Detroit, Mich., U.S.A.) to ensure that the juice was sterile and bacterial colonies did not grow in the media (data not shown).

Table 6.2 shows the effect that cranberry juice has on *Listeria monocytogenes* counts over time. After 30 minutes of incubation in 27% cranberry juice, the *Listeria monocytogenes* counts were reduced to non-detectable levels. The juice treatment that contained 2.7% cranberries had its largest reduction after 9 minutes of incubation with approximately a 2-log CFU/ml reduction. Nogueira et al. (2003) reported similar findings with no growth of *Listeria monocytogenes* after one hour of inoculation with cranberry juice concentrates at Brix of 45- 52.9 g of citric acid/ g juice concentrate. When *Listeria monocytogenes* was held at -11°C in cranberry concentrate, no detectable *Listeria monocytogenes* growth occurred in 39 out of 40 samples (Nogueira et al., 2003).

Figure 6.1 illustrates the effect that the concentration of cranberry juice has on *Listeria monocytogenes* a. At 0.27% and 2.7 % cranberry juice, counts were not significantly different from control levels. However, the sample that had 14% cranberry juice reduced *Listeria monocytogenes* counts by 4 log CFU/ml. The sample with 16% juice reduced counts by 5 log CFU/ml. There was a 6-log reduction in bacterial counts with 19-22% juice. Even though there was a difference in count reduction between 16%
**Table 6.2:** Antimicrobial effect of different concentrations of cranberry juice against *Listeria monocytogenes* for two and half hours in vitro.\(^a\)

<table>
<thead>
<tr>
<th>Cranberry Juice</th>
<th>30 minutes</th>
<th>60 minutes</th>
<th>90 minutes</th>
<th>120 minutes</th>
<th>150 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>10.31±0.12 A</td>
<td>10.36±0.17 A</td>
<td>10.20±0.24 A</td>
<td>10.37±0.17 A</td>
<td>10.20±0.19 A</td>
</tr>
<tr>
<td>0.27%</td>
<td>11.56±0.15 A</td>
<td>11.71±0.09 A</td>
<td>11.02±0.08 A</td>
<td>11.18±0.16 A</td>
<td>11.80±0.17 B</td>
</tr>
<tr>
<td>2.70%</td>
<td>8.7±0.23 A</td>
<td>8.50±0.18 B</td>
<td>8.35±0.14 B</td>
<td>8.27±0.26 B</td>
<td>8.34±0.13 C</td>
</tr>
<tr>
<td>27%</td>
<td>1.7±0.19 B</td>
<td>nd C</td>
<td>nd C</td>
<td>nd C</td>
<td>nd C</td>
</tr>
</tbody>
</table>

\(a\) All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within the same time column followed by the same letter are not significantly different (\(P > 0.05\)) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

nd indicates CFU/ml at non-detectable levels
All analyses were based on three separate experiments. Means followed by the same letter are not significantly different (P < 0.05) from each other. Statistical comparisons of all pairs were analyzed using Student’s t test following one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

Figure 6.1: Antimicrobial effect of various concentrations of cranberry juice at pH 2.5 on *Listeria monocytogenes* after incubation for one hour.
to 22%, the differences were not statically different. The most effective concentrations were 24-27% of cranberry juice. The *Listeria monocytogenes* counts at these high concentrations of juice were reduced to non-detectable levels.

Figure 6.2 illustrates the effect that pH has on the growth of *Listeria monocytogenes*. It was noticed that after 1 hour the bacteria counts were nearly non-detectable (figure 6.1), so in the pH study the time for incubation was set at one hour. Regardless of the pH, treatments were $\geq 1.5$ log CFU/mL lower than the control. From pH of 3 to 11 there is statistically no difference in the reduction of counts between the cranberry juice and the BHI broth. Studies have shown that *Listeria monocytogenes* can grow in a pH range of 5.0-6.0, survive at a pH above 6 and less likely to survive in samples with a pH below 5 (Ryser et al., 1997; Perry and Donelly, 1990). The major difference in the counts between the cranberry juice and the BHI broth occurs at pH 2. It is at this pH that the cranberry juice reaches non-detectable levels and the BHI broth has a greater than 6 log reduce over the control. This observation leads to the conclusion that even though the pH will reduce the counts at such low acid levels with the aid of benzoic acid, there is some synergist relation between the phenols and the benzoic acid in the juice. Zuo et al. (2002) was able to separate through GC-MS 15 benzoic and phenolic compounds in cranberry extracts. It is the phenolic compounds in cranberries that are believed to the principal ingredients responsible for the beneficial effects (Zuo et al. 2002 and Lee et al., 2000).

Figures 6.3, 6.4, and 6.5 show the effects of 27% cranberry juice as a marinate for raw shrimp at various time intervals and temperatures. At 37°C there is no significant
Figure 6.2. The recovery of *Listeria monocytogenes* \( \frac{1}{2} \) a after incubation for one hour in cranberry juice and BHI broth at different pH levels.
All analyses were based on three separate experiments. Means within each vertical column followed by the same letter are not significantly different ($P \geq 0.05$) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

Figure 6.3. The effects of 27% cranberry juice under non-vacuum packaging at 37°C for 30 or 60 minutes on raw shrimp. $^a$
All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P ≥ 0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

Figure 6.4: The effects of 27% cranberry juice under non-vacuum packaging at 4°C for 30 or 60 minutes on raw shrimp.  

\[\text{Log CFU/g} \]

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Control</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>60</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>
differences in the marinate at 30 or 60 minutes (Figure 6.3). However, at 4°C there is a significant difference when the cranberry juice marinate is held for 60 minutes (Figure 6.4). After 24 hours the *Listeria monocytogenes* counts were reduced by 1 log CFU/ml.

This study has shown that cranberry juice is effective for the reduction of *Listeria monocytogenes*. It has also been demonstrated that cranberry juice could be as an addition ingredient used by consumers in a marinating sauce to inhibit *Listeria monocytogenes* on the surface of raw shrimp.
All analyses were based on three separate experiments with each mean ± standard deviation being average of three determinations. Means within each vertical column followed by the same letter are not significantly different (P> 0.05) from each other. Statistical comparisons of all pairs were analyzed using one-way analysis of variance (ANOVA) (SAS Institute Inc., Cary, NC).

Figure 6.5: The effects of 27% cranberry juice under non-vacuum packaging at 37°C or 4°C for 24 hours on raw shrimp. a
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CHAPTER 7

CONCLUSIONS
The food and meat industry is a constantly evolving entity. Processors vigorously work to maintain quality and safety in the products they produce. *Listeria monocytogenes* is a foodborne pathogen of concern to ready-to-eat food processors. This ubiquitous pathogen has the ability to survive and grow over a wide range of temperatures. This research has given processors additional information to effectively maintain and improve their standards of quality while continuing to produce a safe product.

The evaluation of *Listeria monocytogenes* at freezer temperatures on ready-to-eat meat products showed that this foodborne pathogen is highly resistant to freezer temperatures over a three month storage period. Processors especially need to exhibit caution during the first 30 days of frozen storage due to the ability of *Listeria monocytogenes* to increase in growth on the surface of ready-to-eat meat products. It should also be observed that based on the type of fat and fat concentration, the potential for growth of *Listeria monocytogenes* at freezer temperatures varies from product to product. In addition, the type of packaging, vacuum or non-vacuum, varies in the products in the survival of *Listeria monocytogenes* at freezer temperatures.

The use of chitosan as an edible film for the coating of ready-to-eat roast beef demonstrated a reduction in the *Listeria monocytogenes* counts. It was observed that chitosan dissolved with acetic acid has more antimicrobial benefits against *Listeria monocytogenes* that chitosan dissolved in lactic acid. Cranberry juice was shown to have strong antimicrobial activity against *Listeria monocytogenes* in vitro, reducing this foodborne pathogen to non-detectable levels. In addition, cranberry juice can be used as a marinate sauce for the control of *Listeria monocytogenes*. 
The use of acidified sodium chlorite as a processing aid before packaging to reduce post processing contamination showed a significant reduction in *Listeria monocytogenes* counts on the surface of ready-to-eat meat products at refrigerator temperatures. Acidified sodium chlorite at levels of 250 ppm to 1000 ppm showed a greater than 2 log reduction in the products after 30 days of storage at refrigerator temperatures. The acidified sodium chlorite solution maybe used as a dip or spray solution depending on the manufacturing procedures for the product. Acidified sodium chlorite does not alter the desired sensory properties regardless of concentration employed.

All in all, this dissertation demonstrates that there is still a need to study the survival mechanism of *Listeria monocytogenes* in food products. There is also a need to develop additional methods to control or inhibit the growth of *Listeria monocytogenes*. The findings of this study will help food processors control *Listeria monocytogenes* on ready-to-eat products.
VITA

The author is a native of Baton Rouge, Louisiana, and a product of the East Baton Rouge Parish School System. She received her high school diploma from Scotlandville Magnet High School in May of 1993. She later went on to study at Southern University and A&M College in Baton Rouge, Louisiana. At Southern, she received a Bachelor of Science degree in biology while concentrating in microbiology during May of 1997. During this same time period, May 1997, she completed her Honors Thesis. At The Ohio State University in Columbus, Ohio, the author obtained a Master of Science degree in animal science with a focus on meat science in December 1999. The author then returned home to Baton Rouge, Louisiana to attend Louisiana State University. She pursued and received a Doctor of Philosophy degree in food science with an area of concentration in food microbiology and safety.

The author is a daughter of Mr. and Mrs. Roosevelt Beverly and the sister of Kredenna, Katina and Miesha Beverly. She is also the wife of Larry Richardson.