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Control of Listeria monocytogenes in ready-to-eat (RTE) vacuum packaged turkey roll via single or combined use of organic acid salts, ALTA 2341, and electron-beam irradiation

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Control of *Listeria monocytogenes* in ready-to-eat (RTE) vacuum packaged turkey roll via single or combined use of organic acid salts, ALTA™2341, and electron-beam irradiation

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CHAPTER 1. GENERAL INTRODUCTION

Listeria monocytogenes causes an estimated 2500 cases of listeriosis annually with an estimated 20% mortality rate among affected persons, attracting attention as one of the three leading known foodborne pathogens in causing death from foodborne disease. This pathogen is a ubiquitous psychrotrophic bacterium which can grow to high numbers in refrigerated highly perishable foods like meat and poultry products during extended refrigerated storage. It also can tolerate very high salt levels in foods, and grow aerobically or anaerobically. All these characteristics make *L. monocytogenes* a difficult organism to control by the common food preservation methods, which are otherwise useful in controlling pathogenic bacteria in foods.

Several well publicized outbreaks of listeriosis that have occurred from consumption of contaminated ready-to-eat (RTE) meat and poultry products have brought the emphasis on the problem of post-processing contamination of these products, and also on intervention strategies to limit or eliminate the problem.

Considering the life-threatening nature of listeriosis, there is need for post-packaging intervention technologies that can reduce or inhibit *L. monocytogenes* numbers in RTE meats without altering the sensory characteristics of the food.

Food irradiation is a novel technology that is approved for use in poultry products in doses up to 3 kGy to assure microbial safety. This method can be effectively used to reduce the pathogen numbers in a packaged food.

Bacteriocins, naturally occurring products from LAB bacteria, have been shown to inhibit *L. monocytogenes* in various food products. Currently only nisin has been approved for use in foods, but pediocin has shown to possess very good listericidal activity.

Organic acid salts such as sodium lactate and sodium diacetate have also been studied extensively for their ability to control *L. monocytogenes* and they have been shown to be effective alone and especially in combination allowing for better control of the pathogen with use within the permissible levels of these food additives.

No technology when used alone seems to be the answer to the problem of *Listeria monocytogenes* in foods. Hurdle technology encompassing the use of combined antimicrobial interventions can rather be more effective in controlling this pathogen with minimal sensory changes in the treated food product.

To our knowledge no research has been conducted so far to determine the effectiveness of combinations of organic acid salts, a bacteriocin and irradiation to control *Listeria monocytogenes* in an uncured ready-to-eat (RTE) poultry product such as RTE turkey roll. The main objective of this study was to evaluate single or combined use of sodium lactate, sodium diacetate, ALTA™2341, and electron-beam irradiation for controlling *L. monocytogenes* in ready-to-eat turkey roll at 4°C and 10°C.

THESIS ORGANIZATION

This thesis consists of two papers to be submitted to the Journal of Food Protection. Each paper constitutes a chapter and contains the following sections: an abstract, introduction, materials and methods, results and discussions, and references cited. Chapter 4 is a comprehensive conclusion that encompasses the findings from both papers. References are located at the end of each chapter and will follow the format for the Journal of Food Protection.

LITERATURE REVIEW

Genus *Listeria*: *Listeria monocytogenes*

Historical Background

Listeria monocytogenes was first described by Murray et al. in 1926 (Murray et al., 1926). Following investigation of a disease that was causing death in rabbits, he isolated from their blood a bacterium that he believed had not been previously described. He noticed that this bacterium caused large mononuclear leucocytosis, so as a result he decided to temporarily name the bacteria *"Bacterium monocytogenes"*. In 1927 Pirie isolated an organism from gerbils in South Africa that he decided to call "*Listerella hepatolytica*" (Pirie, 1927). Later it was discovered that they had isolated the same bacterium, so Murray and Pirie agreed to name the bacteria "*Listerella monocytogenes"*. Later the name was changed to *Listeria monocytogenes*.

Until 1948, *Listeria monocytogenes* was the only species comprising the genus *Listeria*. Subsequently that year the species *L. denitrificans* was included in the genus (Sohier et al., 1948), followed by *L. grayi* in 1966 (Larsen and Seeliger, 1966), *L. murrayi* in 1971 (Welshimer and Meredith, 1971), *L. innocua* in 1981 (Seeliger, 1981), *L. ivanovii* in 1985 (Seeliger at al, 1984), *L. welshimeri* in 1983, and *L. seeligeri* in 1983 (Rocourt and Grimond, 1983). After Stuart and Welshimer used of a DNA hybridization methods to show a high genomic homology between *L. grayi* and *L. murray* (Stuart and Welshimer, 1973), they proposed that these two species should be merged into only one. *Listeria dentrificans* was also excluded from the genus *Listeria* subsequent to 16s rRNA studies that revealed

differences with the other species of the genus *Listeria* and similiarities with the coryneform bacteria (Rocourt et al, 1987)

Taxonomy of genus Listeria

Currently six species comprise the genus *Listeria*: *L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, and L. grayi.* This classification was based on DNA homology, 16s rRNA sequencing homology, chemotaxonomic characteristics, and multilocus enzyme analysis (Rocourt, 1999).

Morphology and metabolism of genus *Listeria*

Listeriae are small gram-positive rods (0.5μm in diameter and 1-2μm in length), but they can also sometimes appear as cocci with a diameter of 0.5μm. They are nonspore-forming bacteria and they do not form capsules (Seeliger and Bokenmühl, 1968; Rocourt, 1999). Members of this species are motile by means of peritrichous flagella when they are cultured at 20-25°C but they show very weak movement or none whatsoever when grown at 37°C (Galsworthy et al., 1990; Rocourt 1999). They are catalase-positive (some strains were shown to not posses this characteristic) oxidase negative, aerobic, microaerophilic, and facultatively anaerobic. They use the Embden-Meyerhoff pathway to catabolize glucose aerobically or anerobically (Seeliger and Jones, 1986; Rocourt, 1999)

Species identification

Tests like hemolysis, acid production from D-xylose, L-rhamnose, alpha-methyl-D-mannoside, and mannitol are used to differentiate species of the genus *Listeria* (Rocourt et

al., 1983, Rocourt 1999). *L. monocytogenes* and *L. seeligeri* produce narrow zones of ß-hemolysis, *L. ivanovii* produces wide zones of ß-hemolysis, while *L. welshimeri, L. innocua* and *L. grayi* are not hemolytic. Differences in acid production from D-xylose, L-rhamnose, and alpha-methyl-D-mannoside differentiate *L. monocytogenes* from *L. ivanovii* (Rocourt, 1999). The CAMP test is considered as the definitive test for *L. monocytogenes*. The isolate is considered presumptive *L. monocytogenes* if it is CAMP positive with *Staphylococcus aureus* or *Rodococcus equi*, but it does not also determine whether the strain is virulent (McKellar, 1994, Jay, 2000).

Growth characteristics

pH

Optimum pH for growth of *L. monocytogenes* ranges between pH 6 and 8. The minimum and maximum pH in which this pathogen can survive varies by strain, but some strains can survive in pH as low as 4.1 and as high as 9.6 (Jay, 2000).

Temperature

According to *Bergey's Manual of Systematic Bacteriology* (Seeliger and Jones, 1986) the minimum temperature in which *L. monocytogenes* can grow is at 1°C. Growth temperatures for two strains of *L. monocytogenes* serotype 1/2 were shown to be as low as 0.5°C, but the minimum growth temperature of 1.1°C ± 0.3 was calculated as the mean of the minimum growth temperatures for 78 strains of *L. monocytogenes* (Junttila et al., 1988). The maximum temperature in which *Listeria* spp. can grow is about 45°C (Jay, 2000).

Water activity (a_w)

Minimum a_w for growth of *L. monocytogenes* in foods is as low as 0.90 in certain conditions (Lou and Yousef, 1999).

Subtyping of *Listeria monocytogenes*

Serotyping

Thirteen serovars are distinguished within the species *L. monocytogenes*, and the serovars that are more often isolated are 1 and 4, with serovar 4b accounting for 65-80% of all strains. Serovar 4b is more commonly related to outbreaks whereas serovar 1/2 is more commonly found in foods (Jay 2000). Serological typing (serotyping) is used to differentiate strains of *L. monocytogenes* on the basis of the antigenic determinants that are expressed on the bacterial cell surface, including lipotechoic acids, membrane proteins and external organelles like fimbriae and flagella (Graves et al, 1999) The determinants are classified into somatic (O) antigens and flagellar (H) antigens (Seeliger and Hohne, 1979). Serotyping can be valuable in screening groups of isolates during outbreaks, but otherwise has a poor discriminating power compared to other subtyping methods (Graves et al., 1999).

Bacteriophage typing

Phages can be used to type *L. monocytogenes* strains based on the susceptibility of some bacterial strains to particular phages and not to others (Rocourt et al, 1985). This method has a high discriminating power, and allows for rapid and mass screening when outbreaks occur, but more standardization work will have to be done to limit variability in results between laboratories (Graves et al., 1999).

Bacteriocin typing

Curtiss and Mitchell tested a bacteriocin called monocin that can be used to diifferentiate *L. monocytogenes* from *L. ivanovii*. The method showed poor discrimination power (Graves et al., 1999). The discrimination power of this method was improved to a discrimination index of .99 when was used in combination with the phage typing method (Bannerman et al., 1996).

Molecular typing methods

Molecular methods have been recently used to subtype *L. monocytogenes*. This group of methods includes: multilocus enzyme elecrophoresis (MEE), chromosomal DNA restriction endonuclease analysis, ribotyping, DNA macrorestriction analysis by pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA, repetitive element-based subtyping, and DNA-based subtyping. (Graves et al., 1999).

Selected virulence factors of *L. monocytogenes*

The PrfA virulence island harbors the majority of the genes that control virulence factors involved in the pathogenesis process of *L. monocytogenes* (Kuhn and Goebel, 1999). Internalin (InlA) plays an important role in the adhesion of the pathogen in the epithelium of the gut (Gaillard et al., 1991). Listeriosin O (LLO) is believed to be an important virulence factor altogether with phospholipase C, making possible the escape of *L. monocytogenes* from the phagosome in the phagocytes (Kuhn and Goebel, 1999). ActA regulates the cell to-cell spread of the pathogen during invasion of the host cells, making it possible for *L. monocytogenes* to invade new cells (Mounier et al., 1990).

Listeriosis in humans

Various researchers have isolated *L. monocytogenes* strains from fecal material of healthy nonpregnant human carriers. The reported prevalence was especially high in persons who were in continuous contact with food or in laboratory workers (Bojsen-Møller, 1972; Müller, 1990). Listeriosis is often manifested in nonpregnant subjects with underlying immunosuppressive conditions. Invasive disease can occur with complications in patients that are suffering from lymphoreticular malignancies, elderly people under therapy to suppress their immunity, persons infected with AIDS, and in other conditions that lower the natural immunity (Louria et al, 1967; Simpson et al., 1967; Buchner and Schneier, 1968; Bizet et al., 1989; Paul et al., 1994). Typical severe manifestations of the invasive form of the disease include sepsis, meningitis, encephalitis , and endocarditis (Bassan, 1986; Gellin et al, 1991; Slutsker and Schuchat, 1999).

Pregnancy is another high-risk factor favoring the onset of this disease, and listeriosis during the third trimester of the pregnancy is better documented, even though listeriosis may occur in all stages of the pregnancy (Bortolussi, 1990; Slutsker and Schuchat, 1999). In these women, signs that are similar to the common flu can be produced, and the condition is present in about two thirds of the infected subjects (Bortolussi, 1990; McLauchlin, 1990). Transplacental infection of the fetus may occur in pregnant women who are experiencing bacteremia, but also the vaginal route of infection is possible. The intrauterine infection of the fetus could result in stillbirths (Slutsker and Schuchat, 1999). Neonates can become infected with *L. monocytogenes* in utero and express the so called early-onset form of listeriosis with more cases of sepsis then meningitis soon after birth. However the clinical form of the disease that is caused from transmission of the pathogen during passage in the

birth canal or probably nosocomially is manifested weeks after birth with meningitis as a clinical sign (Gellin et al., 1991; Slutsker and Schuchat, 1999). There is also evidence that febrile gastroenteritis can occur in healthy hosts (Schlech, W.F., 1997). Serotypes 1/2a, 1/2b, and 4b are associated with more than 95% of human listeriosis cases (Graves et al., 1999).

Outbreaks

From early August 1998 to January 6, 1999, a multistate outbreak involving rare isolates of serotype 4b which shared an unusual pattern by ribotyping methods and pulsed-field gel electrophoresis, caused 50 illnesses and 8 deaths, including 2 stillbirths. Four months after the initial start of the outbreak, the strain involved was isolated from an open package of hot dogs. The company issued a voluntary recall (CDC, 1998). From May 2000, four deaths, three miscarriages and twenty-nine sicknesses were linked to the consumption of contaminated deli meats that included turkey products (CDC, 2000). Eight perinatal and twenty-one nonperinatal cases were reported. On December 12, Cargill Turkey Products, Inc. (Waco, Texas) ceased marketing ready-to-eat foods and two days later issued a voluntary recall for processed turkey and chicken deli meat that could have been contaminated. The latest outbreak occurred in the Northeastern United States causing 46 cases, 7 deaths and 3 miscarriages in eight states (CDC, 2002). The outbreak was linked to sliced turkey deli meats marketed by the company Pilgrim's Pride Foods, which recalled 27.4 million pounds of poultry products. *L. monocytogenes* was isolated from one intact food product, and from 25 environmental samples. The food isolate was different from the one isolated from the clinical cases, but two of the environmental samples shared indistinguishable PFGE pattern to the outbreak isolates.

Distribution of *Listeria monocytogenes*

L. monocytogenes in the natural environment

L. monocytogenes are ubiquitous bacteria that can be naturally found in soil, water, and plants, especially those that are decaying (Rocourt and Seeliger, 1985). *L. monocytogenes* has been isolated from soil, and it has been demonstrated that damp soils with higher moisture content are more likely to be a good survival environment for this organism (Welshimer, 1960; Fenlon et al., 1996). According to *Fenlon* (1999), and based on the evidence of studies on the environmental distribution of *L. monocytogenes*, it can be concluded that soil is not a natural reservoir for this pathogen, but more likely contamination can occur from one of the following sources: a) during harvesting of the grass from the sheath area, b) decayed plant materials, and c) contamination of the soil by animal manure. Contamination of the vegetation seems to occur during harvesting, because far higher numbers of *L. monocytogenes* have been found in harvested plants compared to the plants that have not yet been harvested, linking the contamination again with the presence of the pathogen in the sheath area of the plants (Weiss and Seeliger, 1975; Farber et al., 1989; Fenlon et al., 1996).

Animal feeds have been implicated in harboring *L. monocytogenes.* Silage has been shown to be a good environment for survival and multiplication of these bacteria, especially when the quality of this animal feed was low and pH>4.5 (Grønstøl, 1979). Infiltration of air in non properly baled silage can create a good growth environment for mold, which subsequently can cause a rise in pH of the silage and make it possible for *L. monocytogenes* to grow to high numbers (Fenlon, 1986). *Listeria monocytogenes* can survive and multiply

for years in silage when pH rises to an unacceptable level (Dijkstra, 1971; Fenlon et al., 1996).

Fecal material is a reservoir for *L. monocytogenes*. Gray and Killinger (1966) state that *L. monocytogenes* can be found in the feces of 37 mammals. Humans can be symptomatic or asymptomatic carriers, with shedding rates of 1.8%-9.0% among healthy individuals (Ralovich, 1984). Consumption of contaminated feed has been linked to the presence of *L. monocytogenes* in animal feces, where lower incidences of excretion of the pathogen were observed in grazing sheep and cattle, compared to they ones that were fed silage (Fenlon et al. 1986; Low et al., 1995). Some increase in the excretion rates of *L. monocytogenes* in stressed animals has been showed by some authors (Ralovich, 1984; Fenlon et al., 1996). Several studies have addressed the spread of *L. monocytogenes* in water and sewage and found the pathogen to be widespread in those environments (Watkins and Sleath, 1981; Dijkstra, 1982; Frances et al., 1991). To date no documented evidence exists of waterborne transmission of listeriosis in humans, but listeriosis has been experimentally transmitted in sheep (Gray et al., 1956; Fenlon 1999). Transmission of *L. monocytogenes* from sewage and water to certain foods is a possibility. An example of that are the high rates of infection that have been shown in mussels and oysters in ocean areas where contaminated sewage was deposited (Soontharanont and Garland, 1995).

L. monocytogenes in poultry

L. monocytogenes is often found in raw poultry products. Studies conducted by Genigeorgis et al. (1989, 1990) showed that *L. monocytogenes* was present in 12.5%-16% of different cuts in chicken, and in 23.3%-45% of turkey cuts. Ryser at al. (1996) also demonstrated that the

postprocessing contamination is a major factor in the final contamination of turkey products when they found nine different ribotypes of *L. monocytogenes* in retail ground turkey meat. During a study on incidence and sources of *L. monocytogenes* in the meat processing environment Samelis and Metaxopoulos (1999) isolated *Listeria monocytogenes* from incoming materials (turkey necks and breasts), and from the processed ready-to-eat products, and *Listeria* spp. (not identified to species level) was also isolated in the processing environment, tumblers and a meat cutting machine. Those researchers linked the contamination of the fully processed products to the survivors of the heat process, since no listeriae were found in the slicing room. Anyway, the possibility of post-processing contamination from *L. monocytogenes* present in the environment is highly possible, as highlighted by the latest outbreak that was linked to the consumption of sliceable turkey deli meat. The PFGE pattern from the floors and drains in the processing plant was indistinguishable from that of the outbreak strain (CDC, 2002).

Food Irradiation

Brief history

Discoveries of the X-rays by Roentgen in 1895 and of radioactivity by Becquerel in 1896 were the first moments in the history of irradiation. Irradiation inactivation of undesired organisms in foods was performed successfully for the first time in 1921, when Shwartz used X-rays to inactivate *Trichinella spiralis* in pork. The first scientific paper to prove that ionizing irradiation could be an effective way of preserving meats was the one published by Brasch and Huber in 1947. Until 1953, when the Army program on food irradiation was initiated, several studies were conducted on effectiveness of irradiation to

inactivate microorganisms in foods, and on minimizing the organoleptic changes caused by irradiation. Food irradiation became a feasible technology as Cobalt-60 and Cesium-137 could be readily obtained after the World War II. Initial work on food irradiation was conducted by the US Army after President Eisenhower launched the "atoms for peace program", before the program was assigned to the USDA Eastern Research Center in Pennsylvania in 1980 (Josephson, 1983; Diehl, 1995).

Types of irradiation

The emission or propagation of energy through space or through a material medium defines the concept of radiation (Jay, 2000). At least six forms of radiation can be distinguished based on characteristics like wavelength, frequency, and penetrating power, but only gamma radiation, ultraviolet radiation and microwaves have attracted the attention of the food industry (Diehl et al., 1995; Wilkinson and Gould, 1996; Mendonca, 2002). Irradiation refers mainly to any process involving the application of ionizing radiation , including alpha particles, beta rays or electrons, and x-rays generated by machines or gamma rays from radioisotopes. Ionizing irradiation that is of interest for food preservation includes x-rays, beta rays, and gamma rays that have wavelengths of 2000 Å or less and are very energetic (Diehl et al., 1995; Wilkinson and Gould, 1996; Mendonca et al., 2002). Beta rays have poor penetration power and are inadequate for food preservation. X-rays have stronger penetration power than beta rays; however the difficulty in focusing them in foods limits their application in food preservation. The very high penetration power of gamma rays makes them very attractive for use in food preservation (Mendonca, 2002). Gamma rays have approximately 1-2 million electron volts (MeV) of energy and can penetrate materials

with a thickness of about 40 cm. These rays are emitted by radioisotopes such as cobalt-60 and cesium-137. Electrons, which are relatively low in energy, can be accelerated with a linear accelerator or a Van de Graaf generator to produce energy levels of 10 MeV or higher. High-energy electrons can be used to produce X-rays via bombardment of heavy metals such as tungsten. Doses of ionizing radiation that are adequate to produce positive and negative charges in food can be used to inactivate foodborne microorganisms. The irradiation dose applied to a food is the most important factor of the irradiation process. The unit of the absorbed dose used in the past is called a rad. A rad is equivalent to 100 ergs of energy absorbed per gram of irradiated material. The currently used unit of absorbed dose is the Gray (Gy), which is equivalent to 100 rads or the absorption of 1 joule of energy per kilogram of irradiated material. One kilogray (kGy) is equivalent to 100,000 rads (Wilkinson and Gould, 1996; Mendonca, 2002).

Radiation inactivation of microorganisms

Ionizing radiation inactivates microorganisms through both direct and indirect effects. The direct effect is manifested in the damage caused directly to main components of the cell, particularly the DNA and membrane (Ingram et al., 1980; Mendonca et al., 2002). It is caused by high energy imparted by irradiation rays and particles that directly hit the genetic material of the cell, ejecting electrons from the atoms and producing incisions in the sugar phosphate backbone of the DNA. These incisions could be single stranded or double stranded (Mosely, 1989; Dickson, 2001; Mendonca, 2002). The angle in which the photon hits the target DNA determines which of the above mentioned lesions will occur. If the high energy particle collides with adjacent areas or both strands of the DNA it can cause double

strand breaks, which are very difficult for the microorganisms to repair. When this phenomenon occurs, a more lethal effect is generated, but the double strand breakage is twenty times less likely to occur compared to the single strand breakage. The microorganisms might be able to survive and repair small numbers of single strand breaks, but as the amount of single strand breaks increases, it becomes impossible for bacteria to repair them, and mutations and cell death follow (Mosely, 1989; Dickson, 2001).

The indirect effect is caused as a result of the high energy of the ionizing radiation, part of which is lost as food is irradiated, absorbed by the food matrix. Food irradiation might cause excitation of the molecules of the food without the electrons leaving the atoms or the molecules. Also the process of ionization of the atoms and molecules will follow as electrons are ejected from their orbits when part of the energy gets transferred to them. These reactions produce positive charges in the atom or molecules from which the electron was expelled, and negative charges from the free ranging electrons or in other atoms and molecules that could possibly capture these electrons. These atoms or molecules that have an unpaired electron are called "free radicals" and they are very reactive. Free electrons cause further excitations and ionizations as they move to hit other atoms and molecules (Stewart, 2001). Energy can be transferred to water, which is present abundantly in the cells themselves and in the surrounding food matrix, and very reactive species are formed as a result of the radiolysis of water or because of the interactions between these compounds between these reactive species, dissolved oxygen and other food components. Some of those radicals are: hydroxyl radical, aqueous electrons, hydrogen atoms, hydrogen, hydrogen peroxide and solvated protons. Oxidizing agents like hydrogen peroxide and the hydroxyl radical and reducing agents like the hydrogen radicals enhance the killing effect of irradiation

by causing damage to the bonds between the DNA bases in various locations to produce single or double strand lesions as formed via the direct effect of irradiation. Damages to other components of the cell might also occur and these components include cell membrane, enzymes and proteins (Stewart, 2001; Dickson, 2001; Mendonca, 2002).

Factors influencing irradiation inactivation

Irradiation dose

Inactivation of microorganisms depends on the dose of irradiation applied. With the increase in irradiation doses the lethal effect of the treatment also increases.

Numbers and types of microorganisms

Higher numbers of microorganisms are not as easily inactivated compared to smaller numbers of microorganisms. It can also be stated that a higher resistance to ionizing radiation is more common in viruses, and then in decreasing order in sporeformers and vegetative bacterial cells. Gram positive bacteria display higher radiation resistance in comparison to gram negative bacteria, and stationary phase bacteria are more sensitive than exponential phase bacteria. Yeast, mold and parasites are even more sensitive then bacteria to ionizing radiation, and they can be inactivated by use of much lower doses. (Jay, 2000; Mendonca, 2002).

Food composition and preservation methods

Inactivation of microorganisms by irradiation is greatly affected by the composition of food in which they are present. In liquid media microorganisms are more sensitive to ionizing radiation, compared with when they are present in solid foods. The higher protein content has been reported to offer to the microorganisms increased protection against

ionizing radiation, by scavenging free radicals produced from the radiolysis of water (Diehl, 1995; Mendonca, 2002). Prior sensitization or reduction of microorganisms through other processing methods can increase the effectiveness of irradiation. These methods could include: heating, high hydrostatic pressure, acidification and addition of chemical additives to the foods. Increased resistance of the microorganisms can be observed when drying is used as a preservation method, because this processing method lowers the water activity, thus minimizing the production of free radicals from the radiolysis of water (Thayer et al., 1995; Mendonca, 2002).

Temperature

Microorganisms are inactivated more efficiently at ambient temperatures, compared to near subfreezing temperatures. This effect is explained by the fact that less reactive species are produced as the result of the decrease of water activity as less water is available for radiolysis to occur. Also, the free radicals produced will not be able to move freely from the production side to other areas, as the food converts to the frozen state (Taub, 1979; Diehl, 1995; Mendonca, 2002).

Atmospheric gas composition

The radiation resistance of microorganisms increases as a result of the absence of the oxygen in contact with the food (Jay, 2000). *L. monocytogens* was shown to be more resistant to ionizing radiation in vacuum packaged turkey meat, compared to the aerobically or modified atmosphere packaged product (Thayer et al., 1999). Patterson et al. (1988) reported conflicting results on the effects of the presence of oxygen in the radiation resistance of microorganisms, when they showed a decreased sensitivity of *E. coli* O157:H7 to ionizing radiation, when the microorganism was present in aerobically packaged poultry meat,

compared to vacuum packaged and packaged under carbon dioxide. Several studies (Hastings et al., 1986, Patterson et al., 1988, Thayer and Boyd, 1999) have demonstrated that the types of gas present in the packaging also affect the radiation resistance of the microorganisms. Presence or absence of gases like carbon dioxide, nitrogen and oxygen in the packaging has been shown to affect the radiation resistance of microorganisms.

Irradiation of poultry products

Irradiation doses up to 3 kGy were allowed to control *Salmonella* in aerobically packaged frozen and fresh poultry by FDA in 1990 (Anonymous, 1990). Two years later the USDA FSIS approved the use of irradiation in these products, but required that a minimum dose of 1.5 kGy must be applied (Anonymous, 1992). The irradiated packaged poultry products that have been irradiated are required to carry the "radura" logo in their labels, specify that the product has undergone an irradiation process and explain the intention why the process was conducted (Molins, 2001). In 1999 FDA awarded a one-year license for the application of machine generated radiation (electron-beam and X-ray) for inactivation of microorganisms in prepackaged meat and poultry products (Molins, 2001). In 1997, irradiation of fresh meat and poultry products was approved by FDA (Anonymous, 1997) for control of *E. coli* O157:H7 and other pathogens, and two years later USDA FSIS (FSIS, 1999) also approved it. Thus far irradiation has not been approved for use in ready-to-eat meat and poultry products.

Irradiation inactivation of *L. monocytogenes*

Patterson et al (1989) studied the behavior of four strains of *L. monocytogenes* after irradiation at doses of 0. 0.5, 1.0, 2.0, and 2.5 kGy in phosphate buffer and minced chicken meat. D-values of 0.32-0.49 and 0.42-0.55 were exhibited by *L. monocytogenes* on phosphate buffer and on minced poultry meat respectively, depending on the bacterial strains and media used. D-values of 0.364, 0.559 and 0.699 were shown for *L. monocytogenes* in trypticase soy broth supplemented with yeast extract (TSBYE), in chicken breast slurry and in raw ground beef (Gürsel and Gürakan, 1997). An irradiation dose of 2.5 kGy was considered by the authors as sufficient to assure the safety of the meat product for up to 7 days, when the initial contamination level is not more than 10^3-10^4/g. Huhtanen et al. (1988) irradiated seven strains of *L. monocytogenes* in culture media or in mechanically separated chicken meat. D-values ranging from 0.40 to 0.47 and 0.62-1.03 were reported for *L. monocytogenes* in BNT medium and in chicken meat. The authors considered the irradiation at 2.0 kGy as sufficient to destroy 10^4 cells of *L. monocytogenes*. D-values for three strains of *L. monocytogenes* in broth and in raw beef irradiated at 0.75-4.5 kGy have been reported by El Shenawy et al. (1989). All three strains were much more sensitive to radiation in broth compared to raw ground beef, and the D-values ranged from 0.34-0.5 kGy and from 0.51-1.0 kGy in broth and beef respectively. Injury of the survivors was also observed in this experiment. Similar D-values with the previous studies (0.18, 0.21, and 0.44 kGy) have been reported for one of the three strains of *L. monocytogenes* irradiated in phosphate buffer, TSB-YE, and poultry feed (Farag et al., 1990). The authors reported differences between radiation sensitivity of the three strains expressed in their D-values (0.21, 0.35, and 0.46 kGy), and they also reported that they didn't observe any significant difference between electron-beam

and gamma radiation sources. Tarté et al (1996) also reported D-values for *L. monocytogenes* in ground pork irradiated by an electron-beam that were similar to the values that were shown from other studies using gamma radiation sources. For three different strains of *L. monocytogenes* employed in the study, D-values ranging from 0.372 to 0.638 were reported. D-values for *L. ivanovii* and *L. innocua* in minced pork meat were also shown in the study, and they were 0.372 and 0.638.

Thayer and Boyd (1996) studied the influence of the type of suspending meat and temperature on the radiation resistance of several foodborne pathogens including *L. monocytogenes.* It was postulated that the type of suspending meat would not affect the gamma radiation resistance of *L. monocytogenes.* Monk et al. (1994) reported D-values from 0.507-0.610 kGy for a five-strain mixture of *L. monocytogenes* in ground beef with different fat content and irradiated in frozen or refrigerated state. They reported that neither fat content nor temperature influenced the irradiation inactivation of the five-strain mixture of *L. monocytogenes*. A different perspective on the effect of the temperature on the gamma irradiation inactivation of *L. monocytogenes* was expressed by another study (Thayer and Boyd, 1995). In this study a four strain mixture of *L. monocytogenes* was inoculated on beef and held at temperatures ranging from -60°C to 15°C. Intervals of 5°C between -20°C and 5°C were used to calculate the D-values. It was reported that an estimated $10^{2.9}$ more cells would be inactivated by gamma irradiation at a dose of 2.4 kGy at 0°C compared to -20°C. The increase in D-values when temperatures were in increased from -5°C to 20°C was linear. The same group (Thayer and Boyd, 1999) showed that irradiation effectiveness can be increased by manipulating the gas present in the packaging of minced turkey meat. The presence of carbon dioxide in the MAP packaging increased the sensitivity of *L.*

monocytogenes to gamma radiation as compared to when nitrogen was used. Increased sensitivity of the bacterium was also recorded in aerobic packaging as compared to the vacuum or MAP packaged ground turkey meat. Similar results were reported from another study that showed increased resistance of *L. monocytogenes* in ground pork irradiated under MAP packaging compared to when irradiated under aerobic packaging conditions (Grant and Patterson, 1991). Mead et al. (1990) studied the behavior of *L. monocytogenes* in levels of 10^2 and $10^4/cm^2$ on whole poultry carcasses during storage at 5°C and 10°C following gamma irradiation at a dose of 2.5 kGy. They detected the presence of *L. monocytogenes* in only one out of twelve carcasses. Even though no further detection occurred for up to 14 days at 5°C and for up to 5 days at 10°C, in the end of the storage period the bacteria were detected from 38.8% of the carcasses with higher inoculum levels and from 5.6% of the carcasses with lower inoculum levels. This study highlights the need to combine irradiation with other methods of intervention in order to achieve the control of *L. monocytogenes* in refrigerated products, considering the psychrotropic nature of the pathogen. Patterson et al. (1993) showed that the lag phase of the growth of *L. monocytogenes* on poultry meat during storage at 6°C was increased from 1 day in unirradiated samples to 18 days for samples that had received the irradiation treatment at 2.5 kGy. The study concluded that irradiation of meats offers protection beyond the initial reduction in the bacterial load, which extends its effect into storage, as the combination of cell injuries caused from irradiation and refrigerated storage continue inhibiting the growth of the bacteria for some time. In the same study far shorter lag phases were observed for the growth of *L. monocytogenes* at temperature abuse.

Some authors have explored the effectiveness of irradiation combined with other treatments in order to achieve a better reduction of *L. monocytogenes* in foods with minimal

sensory changes. Shamsuzzaman et al. (1992) combined electron-beam irradiation and sous-vide treatments to control *L. monocytogenes* in vacuum packaged chicken breast stored at 2°C. Even though the sous-vide treatment had only little effect on the initial counts of the pathogen, enhanced effectiveness of irradiation was observed at a dose of 2.9 kGy after the sous-vide treatment made *L. monocytogenes* undetectable for 8 weeks of storage. The shelf life of the product was considerably extended for an extra two weeks. Another study showed that the D-values for heat treatment of *L. monocytogenes* are considerably lowered when the heat treatment follows irradiation (Grant and Patterson, 1995). Gamma radiation sensitivity of *L. monocytogenes* in frankfurters was increased as citric acid in various concentrations was used as a surface treatment prior to irradiation of the meat product (Sommers et al., 2003). It can be concluded that the combination of irradiation with other technologies could make irradiation a more efficient tool in controlling *L. monocytogenes* in foods, especially considering the psychrotrophic nature of this microorganism, which makes it possible for it to grow in refrigeration temperatures.

Lactic acid, Acetic acid and their salts

Food-grade lactic acid is produced from the fermentation of refined sucrose and other carbohydrates and is extensively used as a food preservative. It could also be produced synthetically by hydrolysis of lactorintrile. This organic acid has a pKa of 3.86, is very water soluble (density in a 60% solution-1.14) and also very corrosive and difficult to handle. The pH of its aqueous 1 and 10% solutions at 25°C is 2.28 is and 1.75 respectively (Shelef, 1994). This acid is basically used as an acidulant, and the antimicrobial effect depends on the amount of the undissociated form, basically enhanced with the increase of the ratio of the

undissociated form to the dissociated form contributing to the greater antimicrobial activity. The undissociated form can easily pass through the cell membranes and be able to obstruct metabolic processes (Lou and Yousef, 1999). The acid holds the status of GRAS additive (21 CFR 184.1061) for general purposes in foods, and is used as 1-3% solution as a carcass wash to lower the initial bacterial load. It is also used for a variety of other purposes that include lowering pH in cheese curds, improving shelf stability of unsalted butter and mayonnaise, to prevent "ropiness" in bread dough etc (Shelef, 1994). Approval for the sodium (21 CFR 184.1768), potassium (21 CFR 184.1639), and calcium (21 CFR 184.1217) salts of lactic acid has been granted for use in foods as direct food ingredients. Meat and poultry industry employs sodium lactate ($CH_3CHOHCOONa$) and potassium lactate ($CH_3CHOHCOOK$) as humectants and flavor enhancers, in order to improve the water holding capacity and cooking yields. Calcium lactate finds use in the food industry in the prevention of bread "ropiness" and as a firming agent (Shelef, 1994). This review will concentrate on the antimicrobial properties of lactates and in particular the employment of sodium lactate to control *L. monocytogenes* in foods.

Acetic acid is another organic acid that is considered GRAS for miscellaneous and general purposes (21 CFR 184.1005). This acid is also used as an acidulant in the food industry and the inhibition mechanism is similar to that of lactic acid. It has a pKa=4.79 and is also a very corrosive compound. Acetic acid is soluble in water, and is used is used as a flavoring compound that imparts the vinegar-like aroma, mainly in condiments like mustard or mayonnaise (Doores, 1993). Sodium acetate is considered GRAS (21 CFR 184.1721) substance for miscellaneous and general purposes, as is also calcium acetate (21 CFR 184.1185). Sodium diacetate (CH_3COOH. CH_3COONa) is a mixture of acetic acid (40%)

and sodium acetate (60%) that comes as a white powder. It can be employed in foods as a flavoring agent (imparting the "dry vinegar" flavor), acidulant or as an antimicrobial in foods, and it has been approved as GRAS additive (Shelef and Addala, 1994; 21 CFR 184.1754). Sodium diacetate will be addressed in more detail in this review with regard to its antimicrobial properties and inhibition of *L. monocytogenes* in foods. Both lactic and acetic acid contain antimicrobial properties, but they will not be in the focus of this review.

Sodium lactate ($CH_3CHOHCOONa$)

The mechanism of inhibition employed by lactates has not been elucidated in detail, but different ways in which these salts exert their antimicrobial effect have been proposed. Shelef (1994) lists acidification of the cytoplasm, iron chelation, lowering of the water activity, and specific anionic effect as possible answers to this the behavior of lactates. Shelef and Yang (1991) showed that growth of *L. monocytogenes* in tryptic soy broth, beef and chicken could be affected by the addition of sodium or potassium lactate, and demonstrated that concentrations higher than 5% lactates in broth delay growth of *L. monocytogenes*. It was also found that the pathogen was more sensitive to lactate at concentrations of 2.6% when it was present on beef compared to chicken. The lag phase of growth of *L. monocytogenes* in beef was extended for 1-2 weeks at 5°C. No enhanced effectiveness of the lactates as a result of the combination with nitrate or salt was observed. Buncic et al. (1995) showed bacteriostatic effect of 4% sodium lactate on *L. monocytogenes* throughout storage at 4°C in BHI broth. He also noted that no listericidal effect of the organic acid salt was achieved. Chen and Shelef (1992) studied the effect of water activity and lactates on the growth of *L. monocytogenes* strain ScottA in a cooked strained beef

model system that varied in water activity. It was found that sodium lactate lowered the water activity (a_w) of the meat system, and a greater inhibition was achieved by sodium lactate in samples that contained high moisture compared to the ones that had lower moisture. Sodium lactate at concentrations of 4% delayed growth in samples containing >55% moisture, and was completely inhibitory in the same concentration in samples that contained 25-55% moisture. No listeriostatic effect for concentrations of <4% lactate alone was observed, but enhanced inhibition of *L. monocytogenes* was achieved by combinations of lower concentrations of sodium lactate with 2% NaCl in samples containing >55% moisture. Another study to assess the effectiveness of lactates to control *L. monocytogenes* (strain Scott A) was conducted in pork liver sausage, and sodium, calcium and potassium salts of lactic acid were used (Weaver and Shelef, 1993). Even though in the control samples stored at 20°C *L. monocytogenes* grew by 5 log cycles after 10 days, it did not grow by more than 1.4% in samples containing 4% lactate. Furthermore, during storage at 5°C *L. monocytogenes* grew by 4.5 log cycles after 50 days of storage, but only an increase of 0.88 log cycles was observed in samples containing the same concentration of sodium lactate. Better inhibition effect was achieved by combinations of sodium lactate with sodium chloride, and the authors also concluded that the decrease in water activity was not drastic enough to explain the bacteriostatic effect of the lactates. Unda et al. (1991) also showed that when sodium lactate is pumped with the brine into microwave-ready beef roasts, it can offer protection against *L. monocytogenes* during temperature abuse at 25°C for 24 hours. In another study sodium lactate when used alone at a concentration of 2% suppressed growth of *L. monocytogenes* in bologna-type sausage for up to 28 days of storage at 5°C and did not offer any degree of protection compared to the controls during storage at 10°C (Qvist et al.,

1994). Combiniations of sodium lactate with 0.25% or 0.5% glucono-delta-lactone (GDL) showed very good inhibitory effect against the pathogen throughout storage at 5°C or 10°C. The authors concluded that combining sodium lactate with a pH lowering agent could offer a better inhibitory effect compared to sodium lactate alone. In a similar fashion Samelis et al. (2001) also showed that lactate permitted growth of *L. monocytogenes* in vacuum-packaged sliced pork bologna within 20 to 35 days of storage at 4°C when used alone as a dipping solution in concentrations of 5-10%. In another study sodium lactate added to the formulation at a concentration of 6%, which is higher than the concentrations currently permitted by USDA-FSIS, was bacteriostatic or even bactericidal throughout storage at 4°C for 120 days in vacuum-packaged frankfurters (Bedie et al. 2001). The current permissible levels of lactate for use in meat products have been increased by the U.S. Department of Agriculture-Food Safety and Inspection Service to 3% (4.8% of the commercially [60% wt/wt] available compound), as they also have been increased for sodium diacetate to 0.25% as a response to the 1998-1999 outbreaks linked to consumption of processed meats contaminated with *L. monocytogenes* (Anonymous, 2000).

Sodium diacetate ($CH_3COOH. CH_3COONa$)

The antimicrobial properties of sodium diacetate have been investigated by Shelef and Addala (1994). They found that that sodium diacetate (SDA) had higher antimicrobial effect on *L. monocytogenes* compared to acetic acid alone at the same pH (5.0-6.0). The experiment was conducted on *L. monocytogenes* in brain heart infusion broth containing sodium diacetate. A temperature dependence of the minimum inhibitory concentrations was observed, as the lower temperatures aided in the inhibition of the pathogen, basically

decreasing from 35 and 32mM at 35°C and 20°C to 28mM at 5°C. Sodium diacetate at a concentration of 21mM (0.3%) added to ground beef and beef slurry showed a broad range of inhibition against four strains of *L. monocytogenes*, *Escherichia coli*, *Pseudomonas fluorescens*, *Salmonella enteriditis*, and *Shewanella putrefaciens*. The authors attributed the effect of inhibition on the bacteria mainly to the effect of sodium diacetate itself, rather than to the effect of the low pH, even though pH of meat was lowered from 5.6 to 5.2 by the addition of sodium diacetate. Mbandi and Shelef (2001) achieved enhanced inhibition of *L. monocytogenes* in sterile comminuted beef stored at 4°C and 10°C when they used sodium diacetate (SDA) in combination with sodium lactate. It was shown that SDA at a concentration of 0.2% suppressed growth of *L. monocytogenes* in meat more efficiently than sodium lactate (SL) alone at concentrations of 1.8 and 2.5%. The study reported that a synergistic effect was produced by combinations of 1.8% lactate +0.1% diacetate at 5°C. The same group studied the effect of combinations of sodium lactate and sodium diacetate in a ready-to-eat meat (beef bologna) and found similar results with their previous study. They found that both salts delayed growth of *L. monocytogenes* in this product when used alone during storage at 5°C for 60 days, but when used in combination (2.5% and 0.2% sodium lactate and sodium diacetate, respectively) they were listericidal against one strain of *L. monocytogenes* and listeriostatic against a six-strain mixture. The additives also behaved better when used in combination during storage at 10°C (Mbandi and Shelef 2002). Schlyter et al (1993a) studied the effect of sodium diacetate (0.1, 0.3, and 0.5%) on the viability of *L. monocytogenes* in combination with nitrite, sodium lactate, and pediocin in turkey meat slurries. It was shown that very good inhibition could be achieved through use of sodium diacetate, that was bactericidal at 4 and 25°C. Combining sodium diacetate with sodium

lactate enhanced the effect, and very good listericidal effect was produced through combination of sodium diacetate with pediocin (~7 $\log_{10}$ CFU/ml difference compared to the controls). Samelis et al. (2002) showed inhibition of growth of *L. monocytogenes* in frankfurters throughout refrigerated storage for 120 days by using combinations of SL (1.8%) + SDA (0.25%), while sodium lactate alone inhibited the pathogen for only 35 to 50 days. It can be concluded from the literature that both sodium lactate and sodium diacetate can be effective tools to achieve inhibition of *L. monocytogenes* in ready-to-eat refrigerated foods, especially when used in combination.

Pediocin and the Bacteriocins of the Gram-positive bacteria

Brief history

Food preservation by means of lactic acid bacteria has a long history, since fermentation is one of the oldest food processing methods. Products from the lactic acid producing (LAB) bacteria that are of value in food preservation include inhibitory enzymes, hydrogen peroxide, diacetyl, organic acids and bacteriocins (Ray and Daeschel, 1992; Abbe, 1995; Stiles, 1996). Nisin, one of the most studied bacteriocins produced by the LAB bacteria, is actually produced by *Lactococcus lactis*, which is a common starter culture in the dairy industry. It was the first bacteriocin from Gram-positive bacteria to be described as it was discovered in 1928, when Rogers observed the inhibitory effect of *Streptococccus lactis* on *Lactobacillus bulgaricus* (Rogers, 1928). In 1988 the FDA granted GRAS status to nisin, on basis that this bacteriocin can be naturally found in some dairy dairy products because of

the particular production process (Anonymous, 1988). To date that is the only bacteriocin granted GRAS status for use in foods in the U.S.

Classification

Bacteriocins are described as "proteinaceus compounds which kill closely related bacteria" (Tagg et al., 1976). The current classification of bacteriocins from gram-positive bacteria comprises four groups (Klaenhammer, 1993).

Class I bacteriocins (lantibiotics) are small (<5 kDa) peptides that contain the aminoacid lanthionine (Lan). An example of bacteriocins within this group includes nisin.

Class II bactericins are small (<5kDa) peptides that do not contain the amino acid lanthionine. They are heat stable, membrane active peptides that are further divided into three subgroups. Subclass IIa contains peptides with listericidal properties, in which the N-terminal consensus sequence YGNGV is a distinguishing feature. An example of bacteriocins from this group would be Pediocin AcH, PA-1, and PO2. Subclass IIb includes bacteriocins that require two peptides for their activity, e.g. lactococcin G, and subclass IIc contains sec-dependent secreted bacteriocins, e.g. acidocin B.

Class III bacteriocins are large (>30kDa) heat-labile peptides, e.g. helveticin J.

Class IV bacteriocins are complex and contain non-proteinaceus moietes for their activity.

This review will further concentrate on properties of class IIa bacteriocins, and in particular, properties, mode of action and applications of pediocin.

Biogenesis and structure

Pediocins produced by *Pediococcus acidilactici* strains H, PAC1.0, and PO2 are known as pediocin AcH, PA-1 and PO2 respectively (Lou and Yousef, 1999). Luchansky et al. (1992) described all three bacteriocins as being similar, based on the identical DNA fingerprints of the producer strains, and also on the fact that identical restriction enzyme fragments were yielded. Production and excretion of pediocin is a complex process, which is regulated by a structural gene needed for the production of preformed bacteriocins. Another gene that is called the immunity gene encodes for a protective protein that makes it possible for the producer of the bacteriocin to protect itself from the destructive effect of its own product, and then two more genes that encode transportation and excretion through the bacterial membrane, and an accessory protein that aids excretion, respectively (Nes et al., 1996). The preformed bacteriocin is further modified by cleavage of a N-terminal leader sequence to form the maturated end product (Klaenhammer, 1993). Pediocin PA-1 in its mature form consists of 44 amino acids. Characteristic are the two disulfide bonds between two cysteine residues at positions 24 and 44, a conserved hydrophilic N-terminal, a hydrophobic and/or amphiphilic C-terminal, and a net positive charge (Abee et al., 1995).

Mode of action

Pediocin forms pores in the membranes of the bacterial cells, causing leakage of their intracellular materials, including inorganic phosphate (Pi), amino acids, and potassium but not ATP (Bhunia et al., 1991; Chen and Montville, 1995). Chikindas et al. (1993) showed that the formation of pore complexes in the bacterial membranes by pediocin was not affected by the membrane potential. This bacteriocin causes complete dissipation of the pH

gradient (ΔpH) and partial dissipation of the transmembrane potential (Δ ψ) (Bruno and Montville, 1993; Chikindas et al., 1993). Anyway, ATP in bacterial cells affected by pediocin is depleted by high rates, mainly because the cells strives to maintain their ΔpH and Δ ψ in their normal parameters, and not largely because of the depletion of the inorganic phosphate as a result of leakage from the pores. In general, the mechanism by which pediocin exerts its bactericidal effect seems to be associated with dissipation of the proton motive force (PMF) (Chen and Montville, 1995).

The 'barrel-stave' model has been proposed in order to explain the mechanism by which pediocin attaches to the bacterial cell membrane and forms pores. The initial stage of the mechanism seems to divide authors on the whether or not pediocin receptors are present in the bacterial cell. Some authors have stated that despite the electrostatic interaction, a membrane-bound receptor must be present in the cell for the bacteriocin to be able to attach in the membrane (Bhunia et al. 1991; Chikindas et al., 1993; Abee et al., 1995). The other theory favors pure electrostatic interactions between the positively charged N-terminal of the bacteriocin and the negatively charged phospholipid groups of the membrane lipid bilayer (Chen et al., 1997). The first step of attachment is followed by a destabilization of the bacterial membrane by hydrophobic interactions between a domain in the C-terminal of the bacteriocin and the acyl chains inside the membrane, beginning the destabilization of the cell membrane and increasing permeability (Fimland et al., 1996; Chen et al., 1997). This C-terminal is believed to contain a cell specific region (Fimland, 1996). The 'barrel-stave' model of inhibition by which Class IIa bacteriocins are thought to operate is schematically expressed in Fig. 1.1 (See p. 37).

Biocontrol of *L monocytogenes* in meats by use of pediocin.

Several authors have investigated the use of pediocin to inhibit *L. monocytogenes* in foods with various results. Yousef et al. (1991) showed rapid decrease of 0.74 $\log_{10}$ CFU/ml from the initial numbers of a *L. monocytogenes* in frankfurter exudates held at 4°C within two hours from addition of pediocin AcH. No difference between the bacteriocin treated samples and the other treatments in the study was observed throughout storage at 4°C, even though a steady decrease in microbial number was reported at 25°C. No reduction was observed on *L. monocytogenes* counts when proteinaseK treated pediocin was used. A similar decrease in counts of *L. monocytogenes* (0.6 $\log_{10}$ CFU/unit) was shown in beef muscle by use of pediocin PO2 (El Khaeteib et al., 1993). The bacteriocin showed no residual inhibitory effect after the first initial reduction. This finding agrees with the findings from another study that showed that pediocin AcH had immediate listericidal effect, even causing lysis of some of the strains, but that this bacteriocin had no residual activity (Motlagh et al., 1991). The same group tested the effectiveness of pediocin AcH against *Listeria* spp. in sterile food systems that included raw ground beef. They found that the listericidal effect depended on the strain used, and was observed within the first hour with no bacteriostatic effect occurring during storage at 4 and 10°C (Motlagh et al., 1992). In raw meat inoculated with *L. monocytogenes* a 0.5-2.2 log reduction was reported within 2 minutes from the addition of pediocin (Nielsen et al., 1990). The behavior of *L. monocytogenes* in turkey summer sausage was also studied with pediocin producer or nonproducer *Pediococcus acidilactici* starter cultures added to the meat product. A difference of 2.5 $\log_{10}$ CFU/g in the decrease of the initial numbers was observed, with the pediocin positive strains performing better, and a total reduction of initial bacterial counts of 3.4 $\log_{10}$ CFU/g was achieved

compared to the control (Luchansky et al., 1992). A reduction of 2.6 $\log_{10}$ CFU/g was shown in chicken summer sausage when pediocin positive *P. acidilactici* strains were used to inhibit *L. monocytogenes*, compared to 1.2 $\log_{10}$ CFU/g reduction achieved by pediocin negative strains (Baccus-Taylor et al., 1993). Pediocin-positive or negative strains of *P. acidilactici* were again evaluated against *L. monocytogenes* in wieners at 4°C or at temperature abuse. No pediocin was produced at refrigeration temperature by the pediocin positive strain, and thus no inhibition was observed. A reduction of 2.7 $\log_{10}$ CFU/g was achieved by the bacteriocin producing strain in frankfurters stored at 25°C (Degnan et al. 1992). Berry et al (1991) also observed inhibition of *L. monocytogenes* for up to 60 days in vacuum-packaged frankfurters containing the bacteriocin producing strain JD1-23. Degnan and Luchansky (1992) reported that after only 35 minutes of the addition of pediocin AcH to the meat based slurry not more than 14-40% of the original arbitrary units (AU) could be recovered. They conducted experiments involving the addition of pediocin AcH encapsulated within phosphatidyl-choline-based liposomes in order to minimize binding to meat proteins and inactivation of pediocin by other detrimental factors in a food system. Those same researchers observed a substantial increase in pediocin activity in all slurry systems used in the study, namely 27.5% for the heated muscle and 28.9% for heated tallow respectively. A study to evaluate the effectiveness of pediocin AcH bound to heat-killed *P. acidilactici* cells against *L. monocytogenes* on refrigerated chicken meat showed that a bacteriocin level of 2400 AU/g reduced the levels of the pathogen by 2.8 $\log_{10}$ CFU/g after 28 days at 4°C when the initial inoculum level was 5 $\log_{10}$ CFU/g. When the initial inoculum level of *L. monocytogenes* was 3 $\log_{10}$ CFU/g , the immediate reduction in counts right after the application of the bacteriocin was ~1 $\log_{10}$ CFU/g , and then from day 7 and throughout the

storage study the levels of *L. monocytogenes* remained undetectable. According to the authors, even though pediocin was bound to the meat proteins, and thus no residual pediocin could be retrieved from the chicken meat, the bacteriocin remained biologically active and inhibited *L. monocytogenes* in both raw and cooked chicken meat (Goff et al., 1996). Murray and Richard investigated both nisin and pediocin AcH as possible antilisterial compounds against *L. innocua* as a model organism for *L. monocytogenes* in raw ground pork stored stored at 5°C. It was demonstrated that even though a slight initial reduction in counts was achieved from the use of pediocin, the target organism resumed growth similar to the control after two days of storage. In general it was observed that pediocin AcH didn't perform as well as nisin in a meat system, in that its antibacterial action was pH dependent and it was very quickly degraded to undetectable levels, unlike nisin, which retained partial activity (Murray and Richard, 1997).

Combining pediocin with other technologies to enhance the effectiveness of this bacteriocin against *L. monocytogenes* has also been investigated. Schlyter et al. (1993a) found that even though pediocin alone reduced the counts of *L. monocytogenes* in turkey slurry by 0.9 $\log_{10}$ CFU/unit, it didn't have any extended effect on the pathogen throughout storage at 4°C and 25°C, but when combined with sodium diacetate, the combination became listeriostatic or listericidal throughout storage, depending on the concentration of the organic acid salt. Hanlin et al. (1993) showed that both pediocin and nisin performed better if used in combination, having a greater antimicrobial spectrum. The same research group found that the combination of high hydrostatic pressure, electroporation or heat treatment makes injured cells more sensitive to the application of pediocin, increasing the efficiency of the latter. (Kalchayanand et al., 1994; Kalchayanand et al., 1998).

ALTA™2341

Pediocin has antilisterial properties, but so far it has not been approved for use in foods. The addition of the purified bacteriocins in foods requires FDA approval, or the company can self-affirm it as GRAS, but in that case the company is required to prove that the bacteriocin deserves that status if asked by FDA (Muriana, 1995). ALTA™2341 is a product of culturing of corn syrup solids with LAB, using a food grade process. This product is already commercially available as a white powder and is marketed as a natural, multifunctional food ingredient, shelf life extender and flavor enhancer (Anonymous, 2003). Schlyter et al. (1993b) showed that ALTA and pediocin were similar in their intrinsic listericidal activity in tryptose phosphate broth and turkey slurries. Furthermore it was shown that the combination of ALTA with sodium diacetate was listericidal in slurries containing the antimicrobials. In another study ALTA at concentrations of 0.6% was effective in inhibiting *L. monocytogenes* in queso blanco cheese prepared with acidulants. When used in cheese stored at 4°C in combination with acetic acid, a decrease in counts of *L. monocytogenes* of 0.7 $\log_{10}$ CFU/unit was recorded, but populations of the pathogen remained unchanged in cheese stored at 20°C. Combinations of ALTA with both citric or malic acid as acidulants did not produce the same desirable results. The increase in concentrations of ALTA to 2.5%, combined with acetic acid as an acidulant produced more drastic results against *L. monocytogenes* in queso blanco cheese. During storage of the cheese at 4°C, reductions between 0.7 and 3.3 $\log_{10}$ CFU/unit were recorded as ALTA concentrations used in combination with acetic acid increased from 0 to 2.5%. A 3.5 $\log_{10}$ CFU/unit decrease resulted from addition of 2.5% ALTA to that cheese stored at 20°C for 7 days whereas a 0.5 $\log_{10}$ decrease in counts was observed in cheese that contained only 0.6%

ALTA (Glass et al., 1995). ALTA showed good listericidal effect when used as a surface wash in concentrations ranging 10,000AU to 20,000AU to control *L. monocytogenes* in blue crab (*Callinectes sapidus*) meat, but it didn't show any inhibitory effect during storage (Degnan et al., 1994). Inhibition of *L. monocytogenes* on vacuum-packaged frankfurters was achieved at levels of 1.5-1.6 $\log_{10}$ CFU/g when 3000 AU ALTA were used as a surface treatment, while even higher levels of inhibition (1.8-2.1 $\log_{10}$ CFU/g) were seen as the concentration of ALTA was increased to 6,000 AU, for inoculation levels of 3.40 and 5.20 $\log_{10}$ CFU/g (Chen et al., 2004a). Further inhibition during storage at 4, 10 and 25°C was also achieved through use of both concentrations of ALTA, basically inhibiting growth for 7 weeks, 2 weeks, and 1 day for 4, 10 and 25°C, respectively (Chen et al. 2004a). The same group observed enhanced inhibition of *L. monocytogenes* in the same food product when ALTA was combined with postpacking thermal pasteurization and a synergistic antilisterial effect of the combination of ALTA with electron-beam irradiation (Chen et al., 2004b, Chen et al., 2004c).

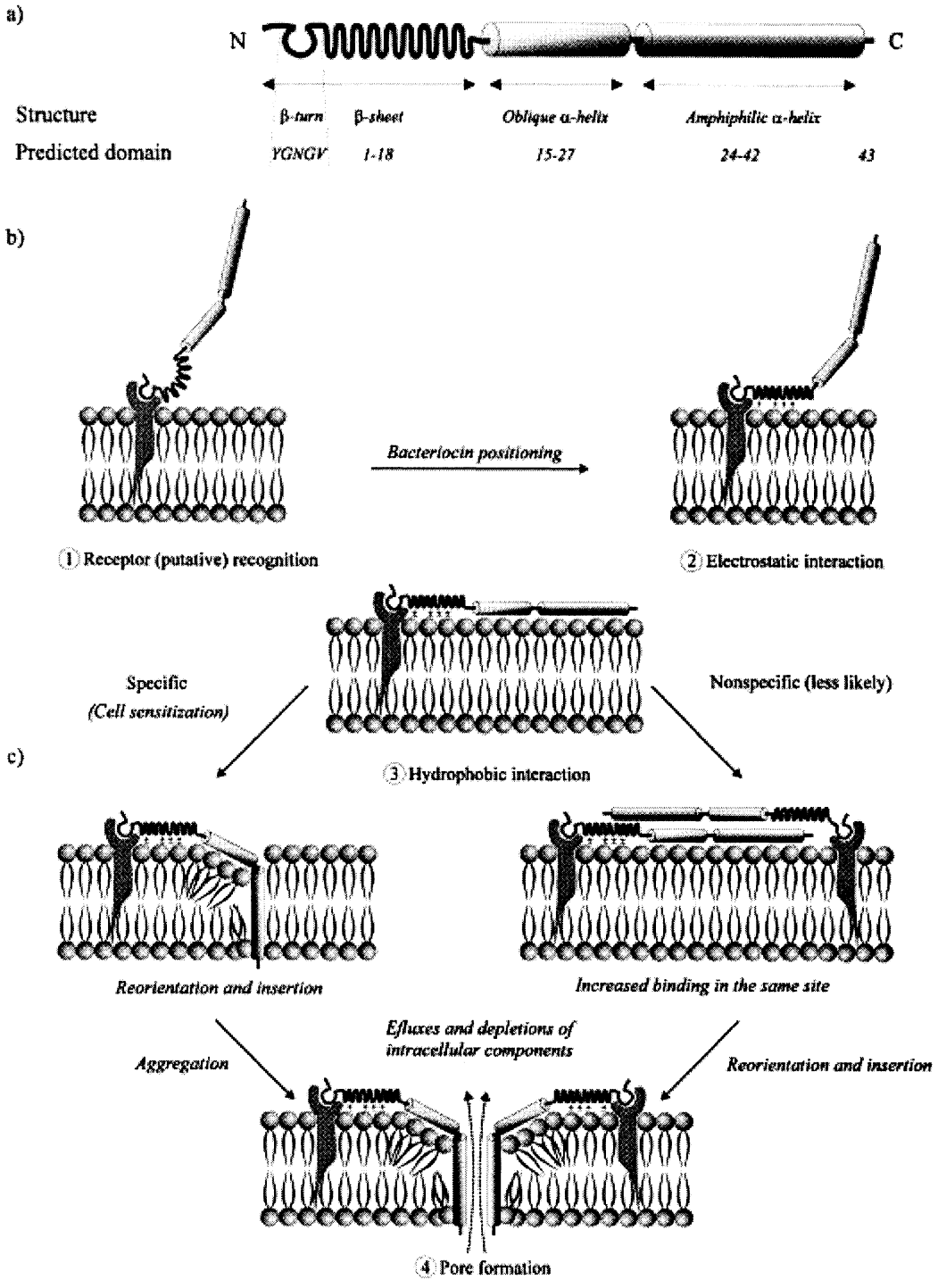

Fig.1.1. Schematic representation of the structure of a model class-IIa bacteriocin and the predicted location its domains with respect to target cell membrane: (a) bacteriocin predicted structural domains; possible interactions of each domain with the membrane surface; bacteriocin insertion and formation of hydrophilic pores. The hydrophobic face of the peptide is shaded dark and the hydrophilic face is shaded light. Adapted from Ennahar et al., 2000.

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CHAPTER 2. EFFICACY OF SODIUM LACTATE AND SODIUM DIACETATE ALONE OR COMBINED WITH PEDIOCIN (ALTA™2341) FOR CONTROLLING *LISTERIA MONOCYTOGENES* IN READY-TO-EAT TURKEY ROLL AT 4°C AND 10°C

A paper to be submitted to the Journal of Food Protection

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ABSTRACT

This study evaluated the efficacy of sodium lactate (SL) and sodium diacetate (SDA) alone or in combination with a commercially available preparation containing pediocin (ALTA™2341) for controlling *Listeria monocytogenes in* vacuum packaged ready-to-eat (RTE) turkey roll. Turkey breast roll was formulated to include SL (1, 2, or 3%) + SDA (0.25%) or ALTA (6,000 AU/g), alone or in combination. The finished product was sliced and inoculated with a five strain mixture of *L. monocytogenes* to give ~10^4 CFU/cm^2. Inoculated turkey roll formulated without SL, SDA or ALTA served as control. All samples were vacuum-packaged and stored at 4°C (42 days) and 10°C (30 days). During storage, populations of *L. monocytogenes* were determined by plating diluted samples onto Modified Oxford (MOX) agar and counting bacterial colonies on MOX agar plates following 48 h of incubation at 35°C. Numbers of *L. monocytogenes* in controls increased rapidly and reached 10^7 CFU/ cm^2 on day 14 (4°C) and day 5 (10°C). ALTA alone did not inhibit growth of *L.*

monocytogenes at 4°C ($P>0.05$) compared to control; however, growth of the pathogen was inhibited at 10°C ($P<0.05$). After 14 days at 4°C the pathogen grew in samples formulated with SL (1%) + SDA (0.25%) + ALTA or SL (1 or 2%) + SDA (0.25%). When ALTA was present in samples with SL (2 or 3%) + SDA (0.25%) no growth of the pathogen occurred for 42 days and 5 days at 4°C and 10°C, respectively. The combination of SL (2 or 3%) + SDA (0.25%) with ALTA (6000 AU/g) has good potential for controlling growth of *L. monocytogenes* in vacuum packaged RTE turkey roll at 4°C.

INTRODUCTION

Listeria monocytogenes is foodborne pathogen of major concern to food processors and public health regulatory agencies because of its widespread distribution in the environment, its ability to grow at refrigerator temperatures, and the high human fatality rate (20 to 30%) associated with listeriosis *(20, 21, 26)*. Most incidences of foodborne listeriosis are usually isolated cases *(25)*; however, in recent years there have been well-publicized multi-state outbreaks of listeriosis involving frankfurters and deli meats *(4, 5, 6)*. More recently, an outbreak in the north east United States was attributed to the consumption of sliceable turkey deli meat. There were 46 confirmed cases, 7 deaths and three still births associated with this outbreak *(7)*. These listeriosis outbreaks have focused much attention on ways to control post-processing contamination of ready-to-eat (RTE) meat products by *L. monocytogenes*.

Several inhibitory treatments including pediocin *(2, 9, 13, 16, 24)* and organic acid salts such as lactates, acetate, and diacetate *(1, 3, 19, 22)* have proven inhibitory to *L. monocytogenes* in RTE products. Goff et al. *(13)* achieved ~1 $\log_{10}$ CFU/g reduction in *L.*

monocytogenes populations in chicken meat immediately after the addition of pediocin to this product. In a recent study *(8)* the application of ALTA to the surface of frankfurters reduced initial populations of *L. monocytogenes* by 2.1 $\log_{10}$ CFU/frankfurter. Synergistic effects of SL and SDA combinations in inhibiting *L. monocytogenes* growth have been demonstrated in frankfurters *(22),* wieners *(12)*, turkey meat slurries *(24),* and beef bologna *(15)*. The incorporation of lactates or acetates, alone or in combination, in the formulation of the cured meats has been shown to exert a bacteriostatic effect on *L. monocytogenes* in these products *(1, 3, 19, 22, 27, 28)*. The current permissible levels of lactates and sodium diacetate for use in meat products are 3% (4.8% of the commercially [60% wt/wt] available compound), and 0.25%, respectively *(11)*.

Certainly, the efficacy of antimicrobial food additives to inhibit growth of *L. monocytogenes* in RTE meats needs to be validated in these products due to differences in product formulation and various levels of antimicrobials used by processors. Preliminary experiments in our laboratory revealed that a combination SL (2%) and SDA (0.25%) consistently failed to suppress growth of *L. monocytogenes* vacuum-packaged RTE turkey breast roll, a non-cured meat product, beyond 21 days of storage at 4°C. These results warranted further research to improve the antilisterial efficacy of lactate and diacetate combinations in RTE turkey roll. To our knowledge there is no published research on the combined effect of lactate, diacetate and pediocin (ALTA™2341) on the growth of *L. monocytogenes* in refrigerated RTE turkey breast roll. Accordingly, the objective of this study was to determine the efficacy of sodium lactate + sodium diacetate alone or in combination with ALTAfor controlling *Listeria monocytogenes* in vacuum-packaged RTE turkey roll.

MATERIALS AND METHODS

Bacterial strains and Culture conditions

A five strain mixture of *L. monocytogenes* (Scott A NADC 2045 serotype 4b, H7969 serotype 4b, H7764 serotype 1/2a, H7762 serotype 4b, and H7962 serotype 4b) was used in this study. A frozen (- 70°C) stock culture of each strain which was maintained in brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI) supplemented with 10% glycerol was activated by aseptically transferring 0.1 ml of the thawed stock culture to 10 ml of tryptic soy broth (Difco Laboratories, Detroit, MI.) supplemented with 0.6% yeast extract (TSBYE) (Difco) followed by incubation at 35°C for 24 hours. Prior to each experiment, each stock culture was transferred twice in TSBYE and incubated at 35°C for 18 h.

Turkey roll preparation

Turkey breast roll was prepared in the Meat Laboratory at Iowa State University. The control product (no added SL, SDA, and ALTA) was formulated to include 0.75% salt and 0.4% sodium tripoly phosphate (STP) (Innophos Inc., Cranbury, NJ) followed by tumbling intermittently for 4 hours. Appropriate concentrations of SL, SDA and ALTA were added to the base formulation during tumbling to give the following treatments: 6,000 AU/g ALTA™2341 (Quest Intl., Sarasota, FL), 1.0% SL (~1.7% of a 60% [wt/wt] commercially available syrup Purac Inc., Linconshire, IL) + 0.25% SDA (Niacet, Niagara Falls, N.Y.); 2.0% SL + 0.25% SDA; 1.0% SL + 0.25% SDA + 6,000 AU/g ALTA; 2.0% SL + 0.25% SDA + 6,000 AU/g ALTA; 3.0% SL + 0.25% SDA + 6,000 AU/g ALTA. Arbitrary units of ALTA used in this study were determined using the "spot-on-lawn" method *(16)*. Twofold dilutions of a solution of ALTA in 10 mM of sodium phosphate buffer (pH 6.5) were prepared, and 10 µl of each dilution was transferred to the surface of a TSAYE plate

containing a lawn of ~10^7 CFU/ml of *L. monocytogenes* Scott A. The arbitrary units (AU) were defined as the reciprocal of the highest dilution showing clear zones of inhibition multiplied by 100, in order to express the arbitrary units per milliliter (AU/ml). Tumbled meat was vacuum stuffed (Risco Model RS 4003-165; Stoughton, Mass.) into large diameter (4.52 inches) fibrous casings, clipped and heat processed in an Alkar oven (Alkar, Lodi, WI) to an internal temperature of 71°C, followed by showering for 15 minutes. Subsequent to cooking, the turkey rolls were chilled (0°C) overnight, sliced (2 mm in thickness), packaged, and transported to the Microbial Food Safety Laboratory.

Inoculation and packaging

Slices of turkey roll were aseptically removed from the original bulk package and repacked (1 slice per bag) into nylon-polyethylene bags (Cryovac ® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100°F, 100% RH /100 sq. in. /24 h; oxygen transmission rate = 3-6 CC at 40°F /m^2 /24 h / 0% RH). Six-milliliter aliquots of each culture were combined in a sterile 50-ml conical tube (Nalgene, Nalge Company, Rochester, NY). The *L. monocytogenes* cells were harvested in a Sorvall Super T21 centrifuge (DuPont Instruments, Willmington, DE) by centrifugation (10,000 × g, 10 min, 4°C), washed twice with sterile 0.1% peptone water (Difco), and serially diluted in 0.1% peptone water to a concentration that would give ~10^4 CFU/cm^2 on each turkey roll slice. Each package of turkey meat was inoculated with 0.1 ml portion of the five-strain *L. monocytogenes* mixture. Each inoculated package was massaged manually for about 1 minute to spread the inoculum over the surface of the meat. All the inoculated packages

were vacuum-sealed to 95 kPa with a Multivac A300/16 vacuum-packaging machine (Sepp Haggenmuller KG, Wolfertschwenden, Germany) and stored at 4°C and 10°C.

Microbiological analysis

Each package was aseptically opened using a sterilized scissors. One hundred ml of sterile 0.1% peptone water was added to each meat sample (surface area ~100 cm^2) followed by manual maceration of the bag from the outside and homogenization of the mixture for 1 min in a Seward Stomacher 400 Lab blender (Seward Ltd., London, England) at medium speed. Serial dilutions of the meat homogenate were prepared in 0.1% peptone water. Aliquots of appropriate serial dilutions were surfaced-plated on Modified Oxford (MOX) agar (Difco). All inoculated agar plates were incubated at 35°C for 48 h. Typical *L. monocytogenes* colonies were enumerated and expressed as $\log_{10}$ CFU/cm^2.

Statistical Analysis

Three independent replications of each experiment were conducted. For each replicate experiment two individual samples (two slices of turkey roll) were analyzed for each treatment on each sampling (n = 6). Microbiological data were converted to $\log_{10}$ CFU/cm^2. Separate analyses of data were conducted for each of the two storage temperatures used in the study. The treatment means were compared by using the general linear model (GLM) procedure of the Statistical Anaysis System software program *(23)*. When analysis of variance (ANOVA) revealed a significant difference ($P < 0.05$) Tukey's Studentized Range (HSD) Test was used to compare the treatment means.

RESULTS AND DISCUSSION

Figure 2.1 shows the influence of ALTA alone or combined with SL and SDA in various concentrations on the growth of *L. monocytogenes* in RTE turkey roll at 4°C. Initial populations of *L. monocytogenes* in control turkey roll reached ~ 10^7 CFU/cm^2 in 14 days. There were no significant differences in the numbers of *L. monocytogenes* in turkey roll with ALTA alone compared to control ($p>0.05$). Turkey roll formulated with SL (1 %) + SDA (0.25%) or SL (1%) + SDA (0.25%) + ALTA inhibited growth of the pathogen for 14 days at 4°C. Growth of the pathogen was inhibited for 21 days in turkey roll formulated with SL (2%) + SDA (0.25%). When ALTA was combined with SL (2 or 3%) + SDA (0.25%) no growth of the pathogen occurred for 42 days.

Pediocin has been reported to reduce initial numbers of *L. monocytogenes* within hours of addition to various meat products *(10, 13, 14, 16, 17, 18, 29)*. Goff et al. *(13)* achieved ~1 $\log_{10}$ CFU/g in initial numbers of *L. monocytogenes* immediately after the addition of pediocin to the chicken meat. Luchansky et al. *(14)*, observed a reduction in the initial counts of *L. monocytogenes* of as high as 3.4 $\log_{10}$ CFU/g compared to the control during manufacture of turkey summer sausage that contained pediocin-producing *Pediococcus acidilactici*. Recently, Chen et al. *(8)* demonstrated a 2.1 log reduction of *L. monocytogenes* on the surface of frankfurters (4°C) following a surface application of ALTA (6,000 AU/frankfurter). In contrast, our results indicate that ALTA (6,000 AU/g) failed to reduce initial numbers of the pathogen in RTE turkey roll (Fig 2.1).

Observed variations in antimicrobial effects of pediocin or pediocin-containing meat additives such as ALTA used in the previously mentioned studies may be attributed to differences in the type of meat product (raw or cooked) and the mode of application of the

antimicrobial. Our addition of ALTA to cubes of raw turkey breast meat most likely predisposed it to the activity of meat proteases and non-specific binding to meat proteins during tumbling of the raw mixture. Murray and Richard *(17)* reported loss of activity of pediocin AcH in raw meat, compared to cooked meat and suggested that proteolytic activity of the natural proteases in meat could be responsible for this phenomenon, especially since these proteases are released during grinding. Yousef et al. *(29)* showed 0.74 $\log_{10}$ CFU/ml reduction of *L.monocytogenes* in frankfurter exudates within two hours from the addition of the antimicrobial, but no effect of the bacteriocin was observed when pediocin treated with proteinase K was used. Degnan et al *(9)* suggested that non-specific binding with meat proteins as well as binding with the dead *Listeria* cells were associated with loss of bacteriocin activity and encapsulated pediocin in liposomes to protect them from being inactivated. Other possible factors that hindered the effect of the ALTA could have also been poor diffusion of the additive in the meat product.

Figure 2.2 shows the effect of ALTA alone or combined with various combinations of SL and SDA on the growth of *L. monocytogenes* in RTE turkey roll at 10°C. Storage of the turkey roll at 10°C was carried out to simulate temperature abuse that these products may sometimes endure during improper storage and distribution. Initial numbers of *L. monocytogenes* increased rapidly in control and reached 7.49 $\log_{10}$ CFU/cm^2 at day 5. All treatments were statistically significant ($p < 0.05$) in inhibiting growth of pathogen; ALTA alone was the least effective treatment. All treatments containing combinations of SL and SDA alone or combined with ALTA inhibited growth of the pathogen for up to10 days. After 10 days. *L. monocytogenes* grew steadily in turkey roll with SL and SDA with or without ALTA and reached ~ 6.0 $\log_{10}$ CFU/cm^2 at day 25. These results are consistent with

those of previous studies that showed faster growth of *L. monocytogenes* in RTE meat products during temperature abuse. Mbandi and Shelef (15) observed greater growth of *L. monocytogenes* in beef bologna stored at 10°C compared to when it was stored at 4°C. Growth of the pathogen after 10 days of storage at 10°C occurred also in most inhibitory treatments that contained 2.5% SL + 0.2% sodium diacetate.

Based on our findings, combinations of SL (1% or 2%) + SDA (0.25%) added in the formulation for RTE turkey roll used in the present study are inadequate to prevent growth of *L. monocytogenes* in this popular meat product during refrigerated storage (4°C) for 42 days. Addition of ALTA (6,000 AU/g) to the meat formulation can enhance the antilisterial efficacy of SL (2%) + SDA (0.25%) in refrigerated RTE turkey roll and ensure the microbial safety of this popular non-cured RTE meat product.

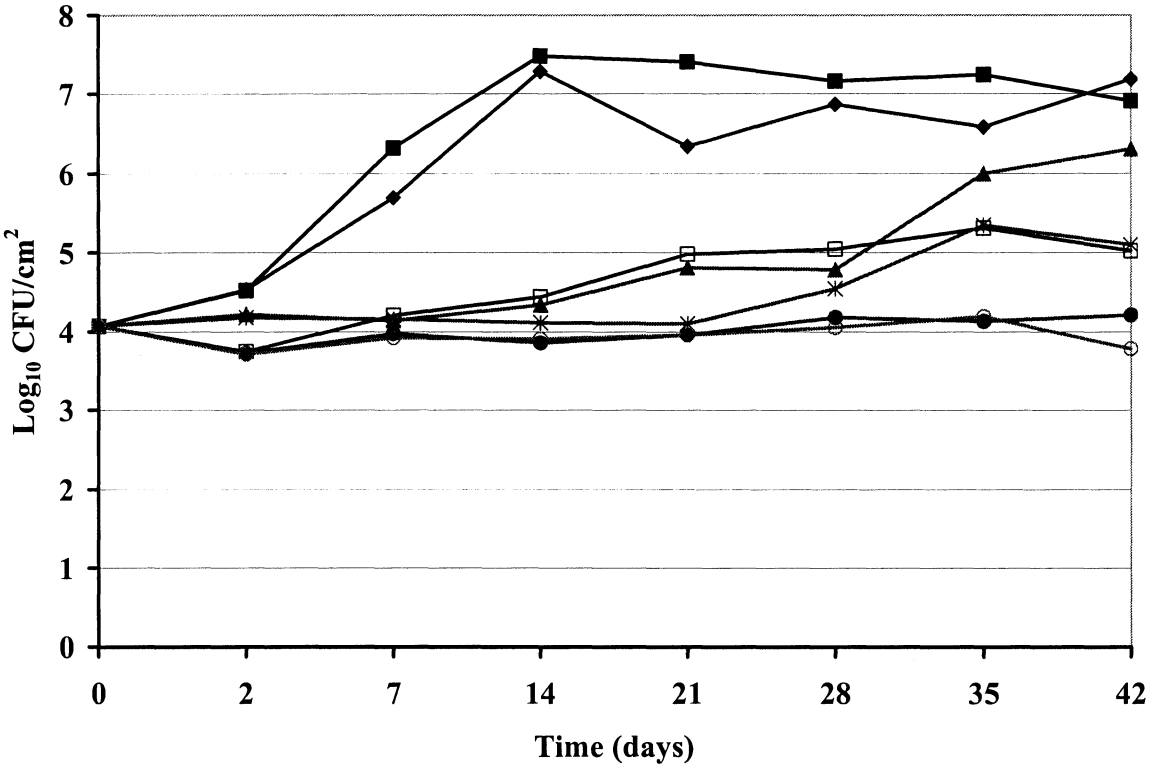

Figure 2.1. Viability of *L. monocytogenes* at 4°C in RTE turkey roll formulated with 1, 2, and 3% SL + 0.25% SDA with or without 6,000 AU/g ALTA™2341. (■) Control, (♦) ALTA 6,000AU, (▲) sodium lactate 1% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25%, (□) sodium lactate 1% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (○) sodium lactate 3% + sodium diacetate 0.25 + ALTA 6,000 AU/g.

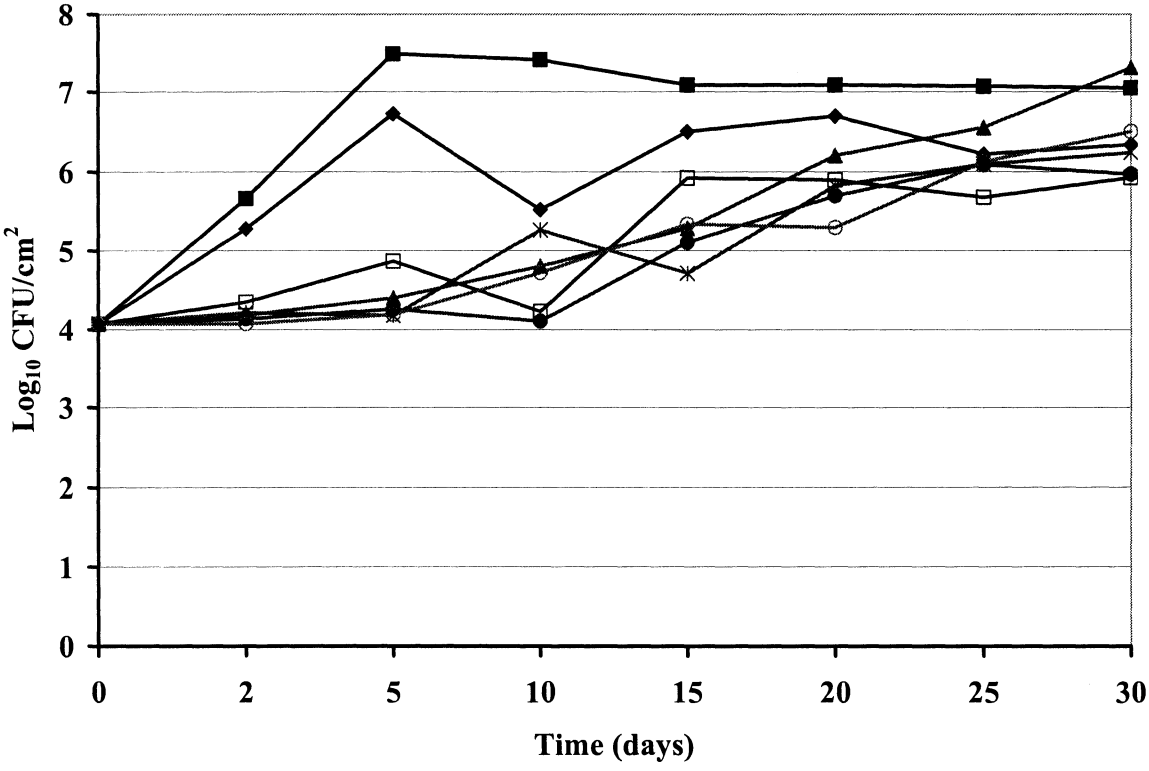

Figure 2.2. Viability of *L. monocytogenes* at 10°C in RTE turkey roll formulated with 1, 2, and 3% SL + 0.25% SDA with or without 6,000 AU/g ALTA™2341. (■) Control, (♦) ALTA 6,000AU, (▲) sodium lactate 1% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25%, (□) sodium lactate 1% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (○) sodium lactate 3% + sodium diacetate 0.25 + ALTA 6,000 AU/g.

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CHAPTER 3. FATE OF *LISTERIA MONOCYTOGENES* FOLLOWING ELECTRON-BEAM-IRRADIATION IN READY-TO-EAT (RTE) TURKEY ROLL CONTAINING SODIUM LACTATE, SODIUM DIACETATE, AND ALTA™2341 ALONE OR IN COMBINATION AT 4°C AND 10°C

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ABSTRACT

The effectiveness of electron beam irradiation for controlling *Listeria monocytogenes* in ready-to-eat (RTE) turkey roll, formulated with ALTA™2341 (6,000 or 12,000 AU) alone or combined with sodium lactate (SL, 2%) + sodium diacetate (SDA, 0.25%), was investigated. Slices of turkey roll were inoculated with a five-strain mixture of *L. monocytogenes* to give ~10^6 CFU/cm^2. Inoculated product without ALTA™2341 or SL + SDA served as controls. Meat samples were vacuum-packaged then irradiated at 0, 1.0, 1.5, 2.0, and 2.5 kGy. Samples irradiated at 0, 1.5, 2.5 kGy were stored at 4°C (42 days) or 10°C (30 days). *L. monocytogenes* were enumerated by plating serial dilutions of meat homogenate on Modified Oxford (MOX) agar and counting bacterial colonies on agar plates after incubation (35°C, 48h). Irradiation at 1.5 and 2.5 kGy reduced initial populations of *L. monocytogenes* by ~2.4 and~ 4.4 log, respectively, irrespective of product formulation. Irradiation D-values were not significantly affected by differences in formulation ($p>0.05$). Growth of survivors in RTE turkey roll with ALTA (6,000 or 12,000 AU) alone was not inhibited ($p>0.05$). Growth of survivors was completely inhibited in samples with SL + SDA ($P< 0.05$). For example, *L.*

monocytogenes in irradiated (2.5 kGy) turkey roll with SL + SDA + ALTA™2341 were less than 10^2 CFU/cm^2 throughout storage at 4°C or 10°C. In contrast, survivors in 2.5 kGy-treated samples without SL + SDA reached ~10^8 CFU/cm^2 at 20 days (10°C) and 35 days (4°C). Irradiation (2.5 kGy) combined with SL (2.0%) + SDA (0.25%) with or without ALTA (6,000 or 12,000 AU) is effective in reducing initial populations of *L. monocytogenes* in RTE turkey roll and preventing growth of survivors during refrigeration (4°C) and temperature abuse (10°C).

INTRODUCTION

The ubiquitous nature of *Listeria monocytogenes (11)* makes this organism a very likely postprocessing contaminant. *Listeria* spp. and *L. monocytogenes* have been found in poultry processing environment and in incoming raw materials *(22)*. A major listeriosis outbreak linked to the consumption of ready-to-eat turkey and chicken products underlined the importance of postprocessing contamination because an indistinguishable PFGE pattern from the strain causing the outbreak was found in isolates from the plant environment *(6)*. *L. monocytogenes* is a heat sensitive organism *(14)* and even though it can survive mild heat treatments, the temperature reached during cooking of ready-to-eat (RTE) turkey roll is more than adequate to inactivate the microorganism in this food product. The contamination of the product could occur in the later stages of the preparation that include but are not limited to slicing, creating the possibility for entry of the organism in the final packaged product. *L. monocytogenes* can grow very well in sliced deli turkey products during storage at refrigeration temperature and reach to high numbers that could eventually pose a grave threat to consumers *(12)*. Postpacking intervention technologies that include, pasteurization of sous-

vide food products *(29)*, high pressure pasteurization *(8)*, and ionizing irradiation *(17)* have been investigated as ways to reduce the contamination of foods.

Ionizing irradiation has proved to be an effective technology in reducing the contamination of these products with *L.monocytogenes (17)*. However, to meet the zero tolerance requirement that USDA has put in place for *L. monocytogenes* in RTE meat products relatively higher irradiation higher doses might be necessary. The use of higher doses, however, could adversely affect the organoleptic characteristics of these products. Combining irradiation with other technologies for control *L. monocytogenes* in RTE meat products will allow for the use of lower doses of irradiation and the achievement of the safety of the product.

Bacteriocins have good potential for use as food preservatives, and pediocin in particular imparts very good antilisterial activity *(4, 5, 9, 13, 28)*. Pediocin has not yet been approved for use in foods, but a fermentation product that has intrinsic listericidal activity similar to pediocin is commercially available under the brand name ALTA™2341 *(1)*. ALTA has good potential for control of *L. monocytogenes* in foods *(27)*. Irradiation and bacteriocins can effectively reduce the initial bacterial load in a food product *(7)*, but they do not exert a bacteriostatic effect throughout storage at refrigeration temperature or temperature abuse, even though the lag phase for bacteria present in those foods is considerably increased by irradiation *(20)*. The risk of the pathogens surviving the application of irradiation and/or bacteriocins and growing to dangerous levels is imminent. This risk may be reduced by the use of foodgrade antimicrobial preservatives that are bacteriostatic. Organic acid salts at different concentrations have been shown to possess bacteriostatic activity when used alone

in meat products *(30, 31)*, but their activity is further enhanced when they are used in combination *(15, 23)*.

The objective of this study was to evaluate the effectiveness of electron-beam-irradiation in combination with ALTA™2341, with or without sodium lactate and sodium diacetate, for controlling *L. monocytogenes* in vacuum packaged RTE turkey roll during storage at 4°C or 10°C.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

L. monocytogenes Scott A NADC 2045 serotype 4b, H7969 serotype 4b, H7764 serotype 1/2a, H7762 serotype 4b, and H7962 serotype 4b were used in this study. All strains were isolates from the 1998-1999 Bil Mar Foods outbreak (CDC, Atlanta, GA), except for Scott A which was obtained from the National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA. The cultures were kept in stock at -70° in brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI) supplemented with 10% glycerol until used. Preceeding every replication of the experiment, two consecutive 18-hr transfer of each stock culture were made in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) supplemented with 0.6% yeast extract (Difco) (TSBYE) at 37°C. A five-strain mixture of *L.monocytogenes* was obtained by pooling equal amounts of each culture. *Listeria* cells in the 5-strain mixture were harvested by centrifugation (10,000 x g, 10 min, 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Willmington, DE), washed twice in 0.1% peptone water (Difco, Detroit, MI), and resuspended in 0.1% peptone water to be used as the inoculum.

Preparation of turkey roll

Turkey breast roll was prepared in the Meat Laboratory at Iowa State University. The control product (no added preservatives) was formulated to include 0.75% salt and 0.4% sodium tripoly phosphate (STP) (Innophos Inc., Cranbury, NJ) followed by tumbling intermittently for 4 hours. Appropriate concentrations of sodium lactate, sodium diacetate and ALTA™2341 were added during tumbling to the base formulation to give the following treatments: 1) Control [without added antimicrobials] 2) ALTA 6,000 [ALTA™2341 6,000 AU/g (Quest Intl., Sarasota, FL)], 3) ALTA 12,000 [ALTA™2341 12,000 AU/g], 4) SL 2% + SDA 0.25% [sodium lactate (Purac Inc., Linconshire, IL) + 0.25% sodium diacetate (Niacet, Niagara Falls, N.Y.)], 5) SL 2.0% + SDA 0.25% + ALTA 6,000 [sodium lactate 2% + 0.25% sodium diacetate + ALTA™2341 6,000 AU/g], SL 2.0% + SDA 0.25% + ALTA 12,000 [sodium lactate 2% + 0.25% sodium diacetate + ALTA™2341 12,000 AU/g]. The fat, moisture *(2)*, and protein content *(3)* of the finished product were measured and the results are presented in Table 3.2. All mixtures were vacuum stuffed (Risco Model RS 4003-165; Stoughton, Mass.) into large diameter (4.52 inches) fibrous casings, clipped and heat processed in an Alkar oven (Alkar, Lodi, WI) to 71°C internal temperatures, followed by showering for 15 minutes. Subsequent to cooking, the turkey rolls were chilled (0°C) overnight, sliced (2 mm in thickness), and packaged for storage at 4°C.

Measurement of pH and water activity (a_W)

The pH of the turkey roll samples was measured by directly inserting the glass electrode of an Orion Aplus pH meter (Thermo Electron Corp., Beverly, MA) in 1:10 slurry of non-inoculated turkey roll. The water activity of the product containing the different treatments was measured using an AQUA LAB a_w meter (Aqua Lab, Pullman, WA).

Measurements were conducted in duplicate at each sampling day along with the microbiological sampling.

Sample inoculation and packaging

Single slices of turkey roll were aseptically transferred into nylon-polyethylene bags (Cryovac ® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100°F, 100% RH/100 sq. in. /24h; oxygen transmission rate = 3-6 CC at 40°F/m^2/24h/0%RH). Each slice was inoculated with 0.1 ml of the five-strain mixture inoculum to give ~10^6cells/cm^2, and later massaged slightly for 1 minute to homogenously spread the inoculum over the surface of the slice. Subsequently, a Multivac A300/16 vacuum-packaging machine (Sepp Haggenmuller KG, Wolfertschwenden, Germany) was used to vacuum seal all samples to 95 kPa.

Irradiation treatment and dosimetry.

Inoculated vacuum packaged slices of turkey roll were irradiated at Iowa State University using the Linear Accelerator Facility, which has a MeV CIRCE III Linear Electron Accelerator (MeV Industrie S.A., Jouy-en-Josas, France). Samples (4°C) were irradiated at five target doses (0, 1.0, 1.5, 2.0, and 2.5 kGy). The procedure was conducted at an energy level of 10MeV at an average dose rate of 70.4 kGy/min. The absorbed doses were determined by using 5mm (diameter) by 5 mm (length) alanine pellets (Bruker Analytische Messtechink, Rheinstetten, Germany) placed on top and bottom of bags containing samples. Measurement of the absorbed doses was conducted immediately after irradiation by inserting the pellets in a Bruker EMS 104 EPR Analyzer (Bruker Analytische Messtechnik, Rheinstetten, Germany), which uses electronic paramagnetic resonance to conduct the analysis.

Microbiological analysis.

Following irradiation, samples were kept in ice for not more than 2 hours before analyzed or placed in storage at 4 and 10°C. Determination of the survivors was performed by conducting analysis of duplicate samples that had been subjected to irradiation doses of 0, 1.0, 1.5, 2.0, and 2.5 kGy. Samples that were exposed to irradiation doses of 0, 1.5, and 2.5 kGy were placed in storage at 4°C for 42 days and analyzed in duplicate at days 0, 7, 14, 21, 28, 35, and 42, or temperature abused at 10°C for 30 days and analyzed at 0, 5, 10, 15, 20, 25, and 30. Analysis was conducted by adding 100 ml of sterile 0.1% peptone water to each aseptically opened bag containing one slice (~100 cm^2) of turkey roll. The bags were macerated by hand from the outside of the bag and then homogenized in a Seward Stomacher 400 Lab blender (Seward Ltd., London, England). Serial dilutions in 0.1% peptone water were performed, and appropriate dilutions were surface plated onto Modified Oxford Medium (MOX) (Difco). The inoculated agar plates were incubated aerobically at 35°C for 48 hours, and then typical *L. monocytogenes* colonies were counted and expressed as $\log_{10}$ CFU/cm^2.

Calculation of D-values.

D-values (irradiation dose in kGy that reduces the initial bacterial population by 1 log or 90%) were calculated by plotting the $\log_{10}$ CFU/cm^2 survivors versus irradiation dose (kGy) using Microsoft Excel 98 Software (Microsoft Inc., Redmond, WA) *(18)*. Regression analysis was conducted and the best fit line was determined. The calculation of the D-values was carried out by calculating the negative reciprocal of the slope of regression curve.

Statistical analysis.

Three replicate experiments were conducted. During each experiment, two samples per treatment were analyzed for each of the storage temperatures (4° and 10°C). D-values were compared using the general linear model (GLM) procedure of the statistical Analysis Software program (SAS Institute, Inc, Cary, NC) *(24)* and Tukey's Studentized Range (HSD) Test. To evaluate the effect of antimicrobials added to the formulation, growth rates of *L. monocytogenes* survivors were subjected to two-way Analysis of Variance (ANOVA). Holm's adjustment (Westfall and Young) was used when pairwise comparisons were performed. Differences were considered statistically significant at $P< 0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Survival of *L. monocytogenes*

Figure 3.1 shows the survival of the five-strain mixture of *L. monocytogenes* subjected to the irradiation doses 0, 1.0, 1.5, 2.0, and 2.5 kGy. Irradiation was effective in reducing the initial numbers of the pathogen ($p<0.05$). The average reduction throughout treatments after exposure to a dose of 2.5 kGy was 4.4 $\log_{10}$ CFU/cm^2. Similar reduction in counts through use of electron-beam irradiation was achieved by Foong et al. *(10)* who reported ~5-log reduction of *L. monocytogenes* numbers in bologna, roast beef, and turkey meat with and without lactate using non-selective media. Romero et al. *(21)* reduced the initial population of *L. monocytogenes* in vacuum-packaged ground turkey meat through use of electron-beam irradiation at a dose of 2.0 kGy by ~3.5 log.

Radiation D-values that were obtained for the different treatments used in this study are presented in Fig. 3.2. D-values ranged between 0.55 and 0.62 kGy. These D-values were similar to those obtained for *L. monocytogenes* (average D-value = 0.61 kGy) in gamma irradiated vacuum-packaged frankfurters *(25)*. No significant differences were observed between the treatments in their sensitivity to electron-beam irradiation ($p>0.05$). A slight, but not significant ($p>0.05$) increase in D-values was observed as the content of the additives in the turkey roll increased with the different treatments. This increase could be explained by the increase in water activity that was determined for all treatments as 0.988, 0.988, 0.983, 0.980, 0.981, and 0.976 for the Control, ALTA 6,000, ALTA 12,000, SL2% + SDA 0.25%, SL 2% + SDA 0.25% + ALTA 6,000, and SL 2% + SDA 0.25% + ALTA 12,000, respectively. A relationship between the water activity and irradiation inactivation exists, because there is less water available for the radiolysis to occur and thus less free radicals are produced *(32)*. We did not observe an increase in radiation sensitivity of *L. monocytogenes* as a result of the added antimicrobials to the formulation ($p>0.05$). Sommers et al. *(26)* increased the irradiation sensitivity of *L. monocytogenes* in frankfurters by previously dipping them in citric acid solutions with concentrations up to 10%. Similar to our study, Foong et al. *(10)* also did not observe any decrease in radiation sensitivity of *L. monocytogenes* when present in turkey meat with lactate, compared to when it was present in turkey meat without added lactate.

Growth of *L. monocytogenes*

Figure 3.3 depicts the behavior of *L. monocytogenes* in non irradiated turkey roll during storage at 4°C for 42 days. Numbers of the pathogen increased steadily for up to 14 days in the controls and in ALTA 6,000 and ALTA 12,000. Treatments containing ALTA

alone at both concentrations were not significantly different ($P>0.05$) compared to the control in the degree of inhibition that they offered. Possible reasons for the occurrence of this phenomenon have been previously discussed. Good inhibition was achieved in treatments SL2% + SDA 0.25%, SL2% + SDA 0.25% + ALTA 6,000, and SL2% + SDA 0.25% + ALTA 12,000, which significantly inhibited the growth of *L. monocytogenes* compared to the controls ($p<0.05$). Figs. 3.4 and 3.5 represent behavior of *L. monocytogenes* during storage at 4°C following irradiation at 1.5 kGy and 2.5 kGy. Initial numbers of the pathogen were reduced by ~2.4 and ~4.4 $\log_{10}$ CFU/cm^2 following irradiation at 1.5 kGy and 2.5 kGy, respectively. Slower growth in the control samples was observed as they started growing only after day 7, but they eventually grew to 7.62 $\log_{10}$ CFU/cm^2 at 21 days in the samples exposed to the irradiation dose of 1.5 kGy, and to 7.93 $\log_{10}$ CFU/cm^2 at day 35 in samples irradiated at 2.5 kGy. Similar behavior was observed at both irradiation doses for treatments ALTA 6,000 and ALTA 12,000, which did not significantly inhibit *L. monocytogenes* during storage at 4°C. Patterson et al. *(20)* also observed increased lag phase in *L. monocytogenes* when the pathogen was subjected to ionizing radiation in raw and cooked minced chicken meat.

Treatments SL2% + SDA 0.25%, SL2% + SDA 0.25% + ALTA 6,000, and SL2% + SDA 0.25% + ALTA 12,000 inhibited *L. monocytogenes* throughout storage at 4°C ($p<0.05$). Pronounced inhibition was observed in samples containing combinations of ALTA and the organic acid salts, as a negative growth rate was observed for *L. monocytogenes* in turkey roll subjected to those treatments. A slight increase in *L. monocytogenes* was observed towards the end of the storage in samples containing organic acid salts alone. Other studies have shown good potential for inhibition of *L. monocytogenes* through use of combinations of salts

of organic acids. Samelis et al. *(23)* showed that combinations of 1.8% sodium lactate with 0.25% sodium diacetate inhibited *L. monocytogenes* in vacuum packaged frankfurters throughout storage at 4°C for 120 days. Difference in the results with our study might arise because our study involved the use of a non-cured meat product (no added nitrite), with a relatively low content of sodium chloride (0.75%).

Growth of *L. monocytogenes* in non irradiated turkey roll and in samples subjected to irradiation at 1.5 kGy, and 2.5 kGy during storage at 10°C is represented by Fig. 3.6, Fig. 3.7, and Fig. 3.8, respectively. *L. monocytogenes* in the controls grew faster than at 4°C in samples exposed to irradiation doses 0 kGy, 1.5 kGy, and 2.5 kGy, reaching 7.67 $\log_{10}$ CFU/cm^2 at day 5, 7.56 $\log_{10}$ CFU/cm^2 at day 10, and 7.43 $\log_{10}$ CFU/cm^2 at day 20, respectively. Treatments containing ALTA alone did not significantly differ from the controls ($p>0.05$), and *L. monocytogenes* numbers in samples containing those treatments increased in a similar fashion to those of the controls. Our results differed from those obtained in a recent study *(7)* which showed synergistic effects of irradiation when combined with ALTA applied on the surface of fully cooked frankfurters and bacteriostatic effect of the bacteriocin during storage. We postulate that such differences might be attributed to our addition of the bacteriocin to the formulation during tumbling of the raw product, resulting in possible degradation of the pediocin in ALTA by meat proteases and/or non-specific binding to meat proteins.

Inhibition of *L. monocytogenes* by the combinations of organic acids alone was achieved during storage at temperature abuse (10°C) ($p<0.05$), but growth of the pathogen was observed after 10-15 days of storage depending on the irradiation dose. Populations of the pathogen increased and eventually reached ~ 7.30 $\log_{10}$ CFU/cm^2 at 20 days of storage.

The reduced inhibition of *L. monocytogenes* by organic acid salts in meat products during temperature abuse (10°C) has been also reported in other studies *(16)*.

In contrast, turkey roll samples formulated with combinations of organic acid salts and ALTA significantly suppressed growth of *L. monocytogenes* throughout storage at 10°C for up to 30 days ($p<0.05$) at all three irradiation doses. A possible way that these combinations exerted the antimicrobial effect can be explained by the fact that pH was lower in those samples as a results of the addition of ALTA (data not shown). A lowering of pH could create more undissociated species of organic acids that can cross the bacterial membrane more easily and cause growth inhibition of the pathogen.

Based on the results of the present study the combined use of irradiation, organic acid salts and ALTA 2341 is effective for reducing initial numbers of *L. monocytogenes* and preventing growth of survivors during refrigeration (4°C) and temperature abuse (10°C).

Table 3.1. Proximate analysis of ready-to-eat (RTE) turkey roll. **1)** Control, **2)** ALTA 6,000 AU/g, **3)** ALTA 12,000 AU/g, **4)** SL 2% + SDA 0.25%, **5)** SL 2% + SDA 0.25% + ALTA 6,000 AU/g, **6)** SL 2% + SDA 0.25% + ALTA 6,000 AU/g

	1	2	3	4	5	6
Moisture (%)	74.14	73.61	72.97	73.34	72.71	70.83
Fat (%)	0.89	0.74	0.88	0.89	0.97	0.93
Protein (%)	22.79	22.34	21.60	22.12	21.59	21.97

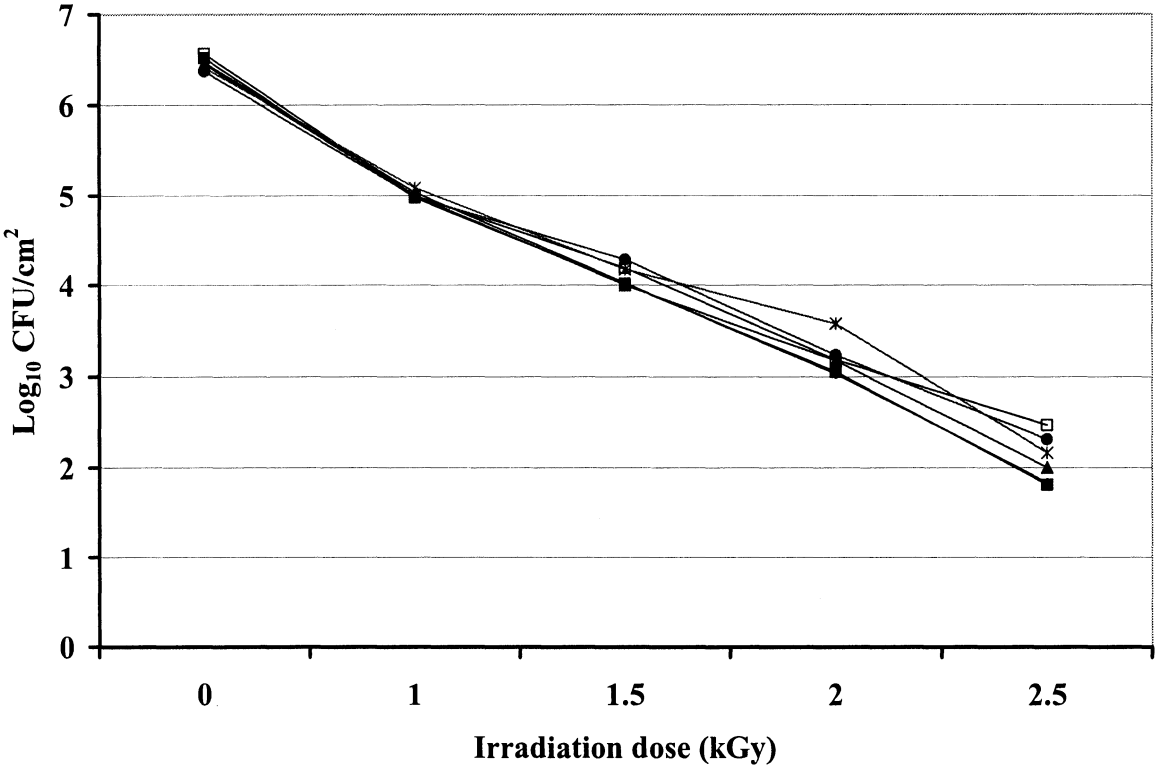

Figure 3.1. Survival of a five-strain mixture of *L. monocytogenes* in RTE turkey roll containing SL + SDA, or ALTA™ alone or combined following electron-beam irradiation. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

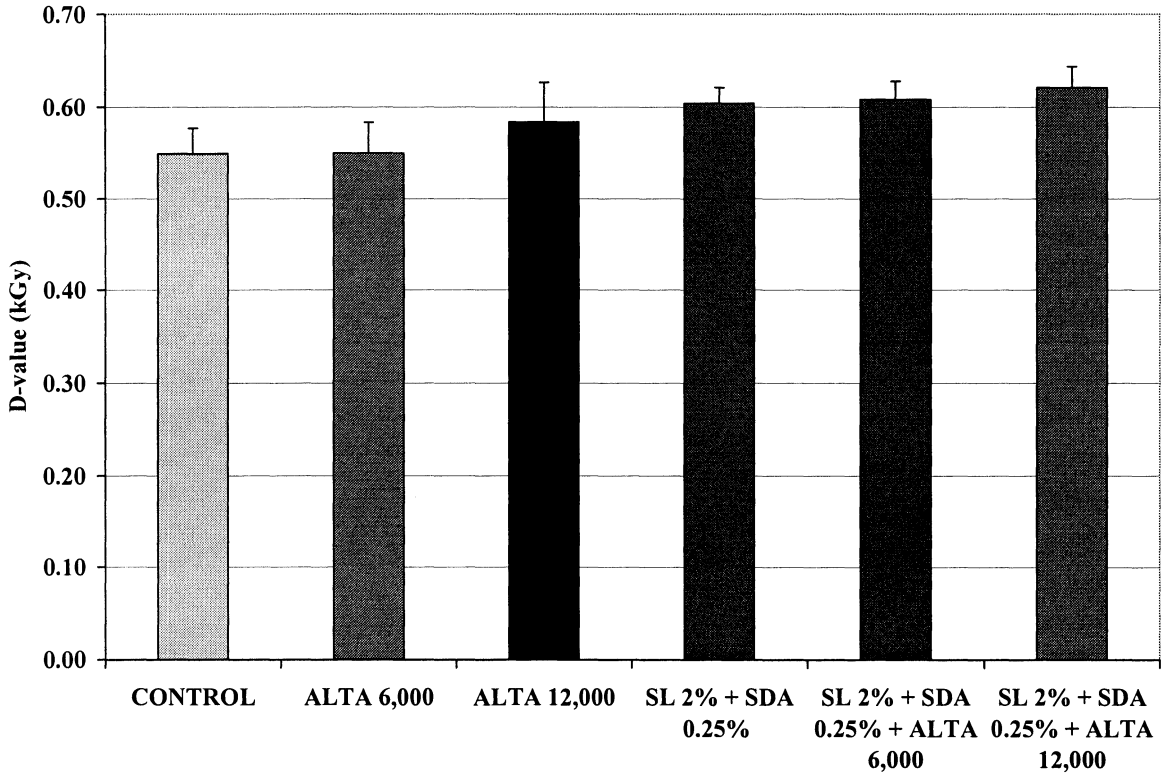

Figure 3.2. D-values for a five-strain mixture of *L. monocytogenes* in RTE turkey roll formulated with SL(2%) + SDA(0.25%) alone or combined with ALTA (6,000 or 12, 000 AU/g). Control contained no added SL, SDA or ALTA.

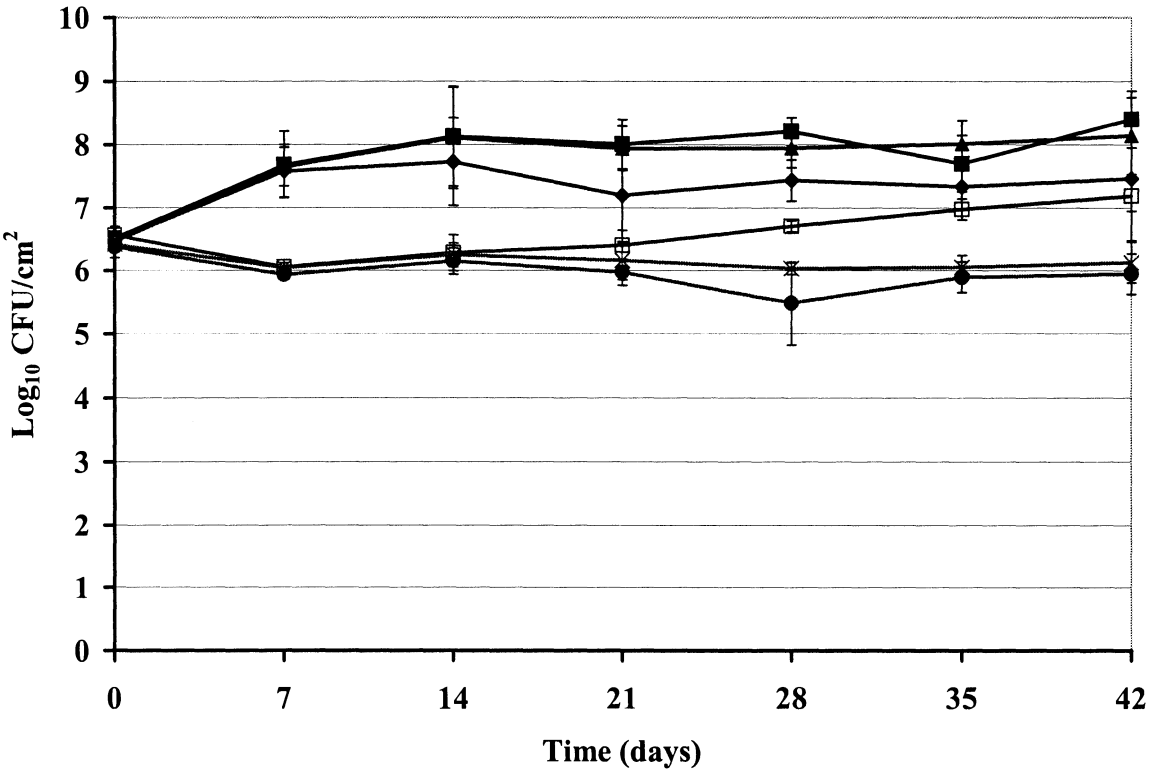

Figure 3.3. Behavior of *L. monocytogenes* in non irradiated RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 4°C. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

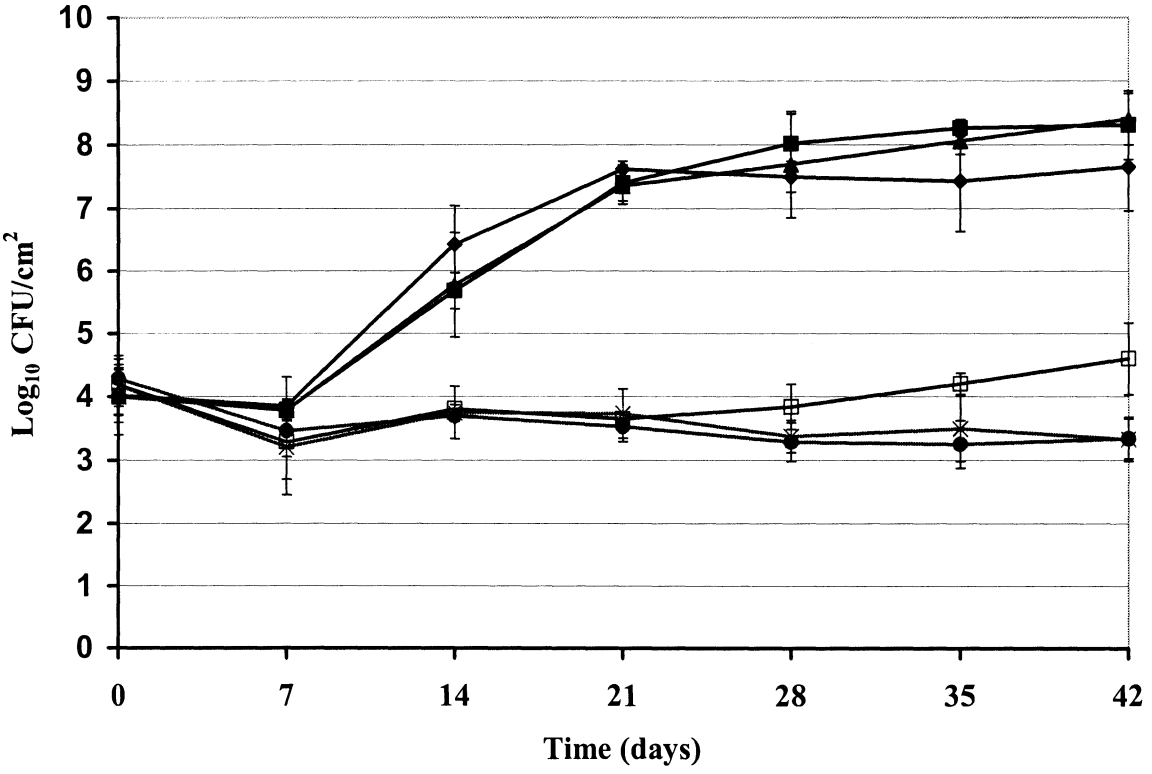

Figure 3.4. Behavior of *L. monocytogenes* in RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 4°C following irradiation at 1.5 kGy. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

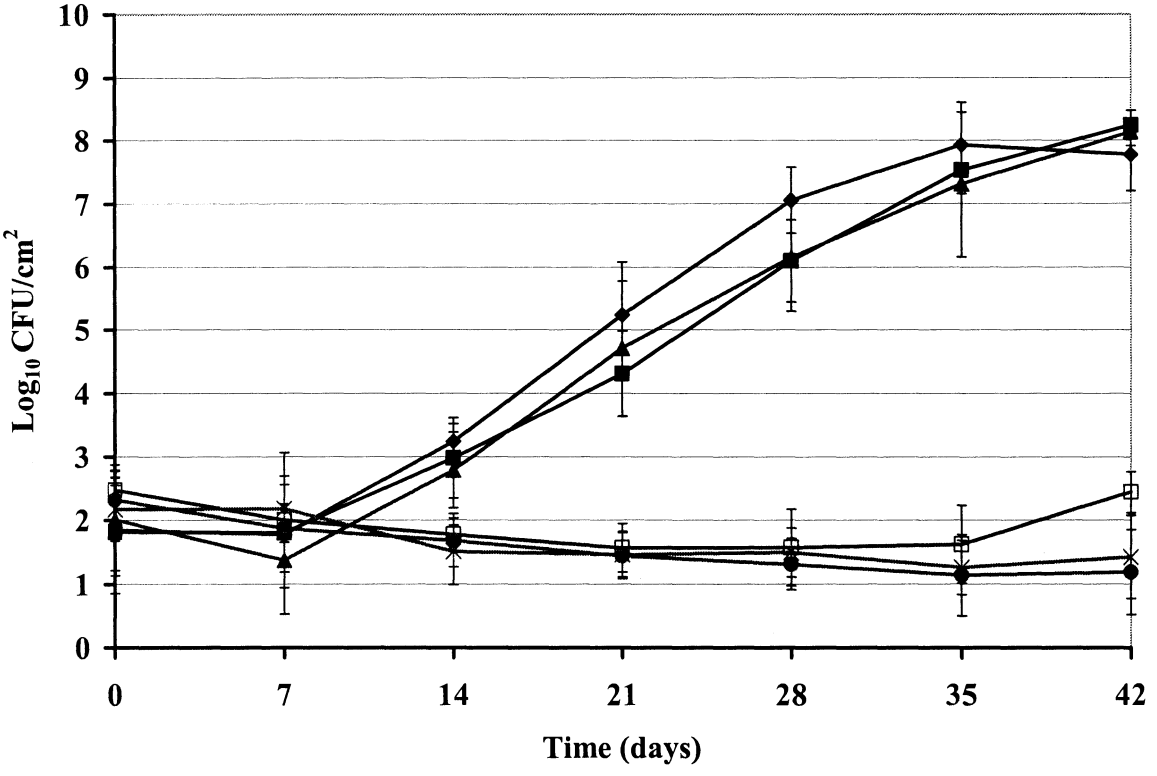

Figure 3.5. Behavior of *L. monocytogenes* in RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 4°C following irradiation at 2.5 kGy. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

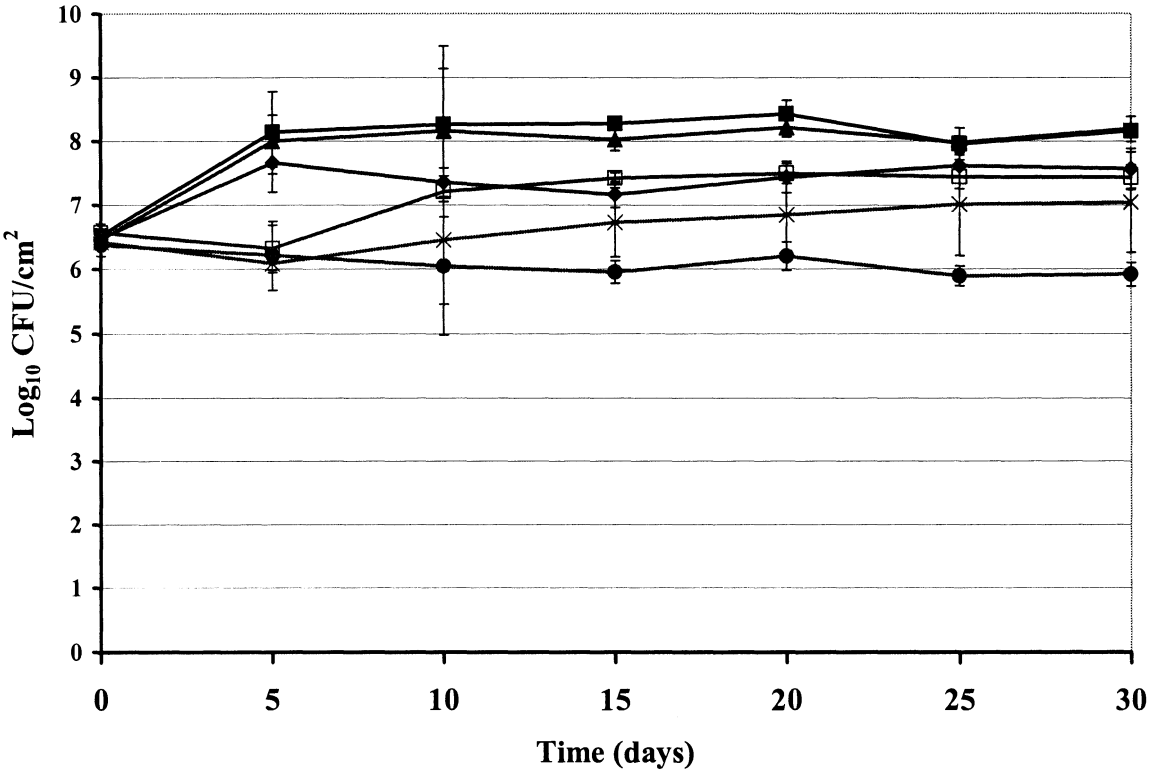

Figure 3.6. Behavior of *L. monocytogenes* in non irradiated RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 10°C. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

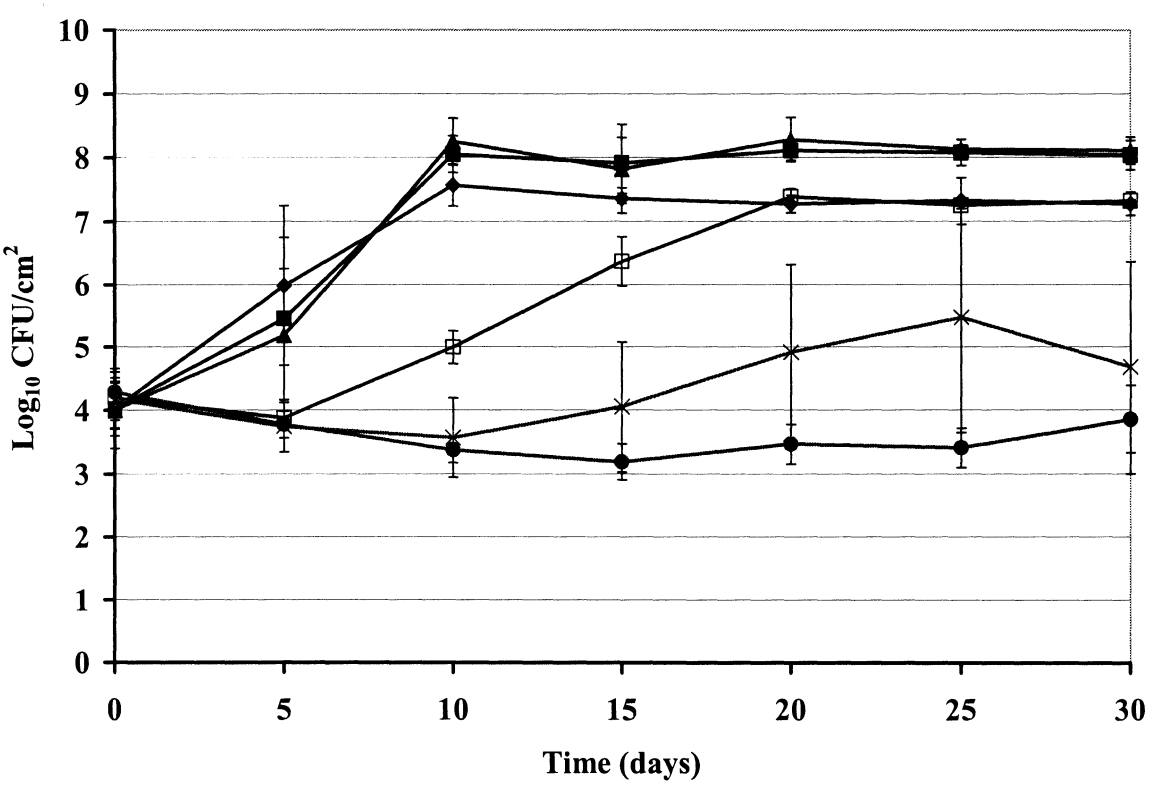

Figure 3.7. Behavior of *L. monocytogenes* in RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 10°C following irradiation at 1.5 kGy. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

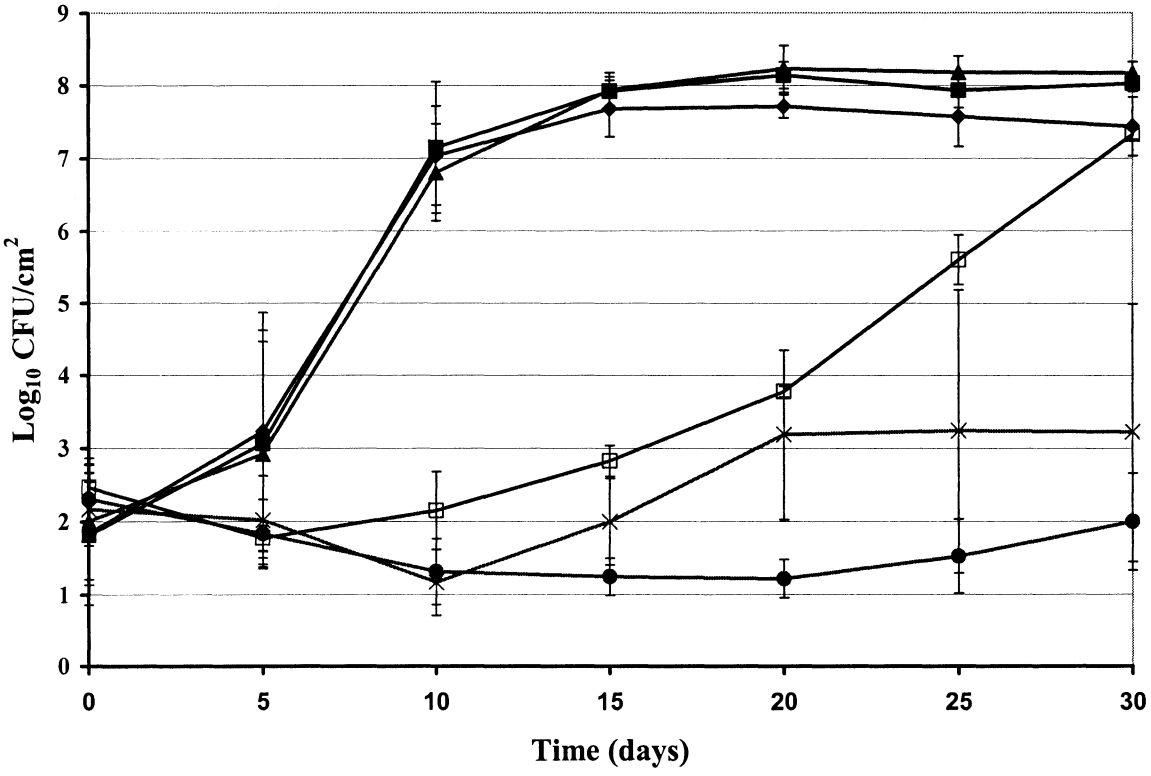

Figure 3.8. Behavior of *L. monocytogenes* in RTE turkey roll containing SL + SDA, or ALTA™2341 alone or combined and stored at 10°C following irradiation at 2.5 kGy. (♦) Control, (■) ALTA 6,000 AU/g, (▲) ALTA 12,000 AU/g, (□) sodium lactate 2% + sodium diacetate 0.25%, (✳) sodium lactate 2% + sodium diacetate 0.25% + ALTA 6,000 AU/g, (●) sodium lactate 2% + sodium diacetate 0.25% + ALTA 12,000 AU/g.

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CHAPTER 4. GENERAL CONCLUSIONS

Electron-beam irradiation proved to be a very effective intervention to reduce the initial *L. monocytogenes* counts in vacuum-packaged ready-to-eat (RTE) turkey roll. Irradiation doses of 1.5kGy and 2.5 kGy reduced the *L. monocytogenes* populations by an average across treatments of ~2.4 and ~4.4 $\log_{10}$ CFU/cm^2. No difference between treatments was observed based on the radiation sensitivity (D-values) of the five-strain mixture of *L. monocytogenes* is concerned. This shows that the direct inactivation of irradiation was more important to reduce the pathogen, compared to the indirect effect. This will allow processors to use these antimicrobials in the formulation of ready-to-eat turkey roll that is to be subjected to irradiation. ALTA™2341 didn't show any listericidal effect when it was used alone in the formulation. Good listeriostatic effect was exerted by some of the combinations of the antimicrobials throughout storage at 4 or 10°C. Combinations of sodium lactate (2% or 3%) with sodium diacetate (0.25%) were found to be effective in inhibiting *L. monocytogenes* during storage at 4°C, but they were not as inhibitory against this pathogen during storage at 10°C. Combinations of sodium lactate (2%) with sodium diacetate (0.25%) and ALTA showed very good inhibition against *L. monocytogenes* in turkey roll stored at this temperature, especially when they were combined with irradiation. It can be concluded that combinations of several intervention strategies consistant with the concept of hurdle technology can assure the safety of foods compared to use of single interventions. Ionizing irradiation, refrigeration temperature, and added chemicals in the formulation can be effective means in controlling *L. monocytogenes* in ready-to-eat turkey roll.

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