Control of Listeria monocytogenes in vacuum packaged frankfurters by generally-recognized-as-safe (GRAS) antimicrobials and electron-beam irradiation

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Control of *Listeria monocytogenes* in vacuum packaged frankfurters by generally-recognized-as-safe (GRAS) antimicrobials and electron-beam irradiation

by

Oleksandr Byelashov

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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Program of Study Committee:
Aubrey Mendonca, Major Professor
Dong Ahn
Joseph Sebranek

Iowa State University
Ames, Iowa
2005

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This is to certify that the master’s thesis of

Oleksandr Byelashov

has met the thesis requirements of Iowa State University

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

*Listeria monocytogenes*, is ubiquitous, gram-positive, psychrotrophic, salt- and pH-tolerant pathogen that can survive in both aerobic and anaerobic environments for long periods of time. Listeriosis, the disease caused by this bacterium, remains an insidious threat to public health and a major food safety problem for the food industry and government regulatory agencies. About 2500 cases of foodborne listeriosis occur each year in the United States resulting in hundreds of deaths and tremendous costs to processors.

Contaminated ready-to-eat (RTE) meats such as frankfurters or deli meats are of major public health concern since they can support the growth of *L. monocytogenes* even when packaged under vacuum and stored at refrigeration temperature. These food products have been implicated in many outbreaks of listeriosis resulting in costly recalls of RTE meats. Proper thermal processing of frankfurters totally eliminates *L. monocytogenes*, but contamination often occurs after cooking, during peeling or packaging of the product. Because of the ubiquitous nature of this pathogen, its ability to proliferate at refrigeration temperature, and the high mortality rate associated with listeriosis (20 to 30%), the United States Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) has mandated a "zero-tolerance" policy for *L. monocytogenes* in RTE meats. Recently, the USDA-FSIS issued an interim final rule requiring additional measures to eliminate *L. monocytogenes* from the products. The second alternative proposed by the rule requires a post-lethality treatment of the product plus antimicrobial agents to suppress bacterial growth. This approach offers flexible options for food producers to control this pathogen, since no single intervention can eliminate *L. monocytogenes* without using levels that may result in a negative effect on quality for these types of products. This alternative uses the concept of hurdle technology, where combined
preservative factors work together by affecting different structural and/or metabolic systems of the microorganism.

Lactic acid (LA), produced by microbial fermentation, is used in the meat and poultry industry for spraying and washing meat carcasses. This organic acid has been consistently shown to inhibit bacterial flora on the surface of RTE meats; when used alone or in combination with sodium lauryl sulfate, it can increase the microbial safety of RTE meat products. The antimicrobial effect of this inhibitory system for use on RTE meats has not been reported. Sodium lactate (SL), the sodium salt of lactic acid, occurs naturally in muscle tissue and has been used to control the growth of spoilage and pathogenic microorganisms, including *L. monocytogenes*, as well as for improving the processing yields and flavor of processed meats.

Sodium lauryl sulfate (SLS), an anionic surfactant commonly used in food industry as an emulsifier, surfactant, and whipping and wetting agent, but it can also be used as a mild antimicrobial. This compound is thought to affect cell membranes, with increased antimicrobial activity at low pH. Also, when used in a dipping, washing, or spraying solutions, it can help to create a uniform film on the surface of the product, providing an even distribution of inhibitory compounds.

Food irradiation can eliminate *L. monocytogenes* from RTE meats, but the high doses required for complete elimination result in undesirable color and odor changes, which make the product less acceptable to the consumer. A combination of low-dose irradiation with generally recognized as safe (GRAS) chemical preservatives may offer a good approach for reducing initial populations of *L. monocytogenes* and controlling the growth of survivors in RTE meats with minimal organoleptic changes in these meat products.

The present study describes the use of a multiple hurdle approach involving antimicrobial treatments applied sequentially or in factorial combinations. Frankfurters formulated with or without sodium lactate were irradiated after a surface treatment with selected antimicrobial solutions that proved to be most inhibitory to *L. monocytogenes*. The objective of this study was to determine the effect of these multiple interventions on the survival and growth of *L. monocytogenes*. Quality
changes in frankfurters were monitored by measuring hunter color values, pH and volatile compounds during product storage.

**Thesis Organization**

This thesis is organized into a literature review (chapter 2), two papers, to be submitted to the Journal of Food Protection (chapter 3&4), and general conclusions (chapter 5). The paper, entitled “Efficacy of Lactic Acid alone or in Combination with Sodium Lauril Sulfate on the inhibition of *Listeria monocytogenes* on Frankfurters made with or without Sodium Lactate” was presented in North Central Branch American Society for Microbiology Annual Meeting in Ames, Iowa on September 22-24, 2005. The second paper “Inhibition of *Listeria monocytogenes* on Vacuum Packaged Frankfurters by Sodium Lactate, Lactic Acid, Sodium Lauryl Sulfate, and Electron Beam Irradiation and Effect on Color and Volatiles” was presented at the Food Safety Consortium Annual Meeting in Manhattan, Kansas on October 2-4, 2005. The references for the thesis were formatted according to the Journal of Food Protection Instructions for Authors and located in the end of each chapter.
CHAPTER 2. LITERATURE REVIEW

Genus *Listeria* and *Listeria monocytogenes*

**Brief history**

The first listeria-like microorganism found in human tissue was reported in by Hayem in 1881 (43). A similar bacterium was observed by Henle in 1893 in Germany and Hulphers in Sweden in 1911, but the full description of this microorganism did not appear until 1926, when Murray and coworkers isolated and characterized it from rabbits at Cambridge University and named it *Bacterium monocytogenes* (62). In 1927 the same bacterium was isolated by Pirie from tissues of gerbils in South Africa and he called it *Listerella hepatolitica* in honor of Lord Lister (97). When it became clear that it was the same microorganism, Murray and Pirie agreed to call it *Listerella monocytogenes*. Later, in 1940, Pirie proposed its renaming to *Listeria monocytogenes* (84). By 1981, *L. monocytogenes* was considered mainly as an animal pathogen, even though there were sporadic cases of listeriosis in humans (62). Currently, *L. monocytogenes* is classified as an “emerging pathogen” due to multiple outbreaks of listeriosis.

**Genus of *Listeria* and taxonomy**

Before 1948 *L. monocytogenes* was the only known species within the genus *Listeria*. Since then, five other species: *L. grayi, L. innocua, L. ivanovii, L. welshimeri,* and *L. seeligeri* have been included in the genus. These species are differentiated by DNA homology, 16S rRNA sequencing homology, and multilocus
enzyme analysis. There are two lines within the genus due to evolutionary
differentiation: *L. grayi* belongs to one line, and the other line consists of the other
five species *L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L.
monocytogenes.* The second line can be further divided into two groups: *L.
monocytogenes and L. innocua* are in group a, and *L. ivanovii, L. welshimeri,* and *L.
seeligeri* comprise group b (17,91,92). Only one of the six species, *L.
monocytogenes,* is considered to be pathogenic to humans.

**Identification and metabolism of Listeriae**

*Listeria* spp. a small (0.5x1.0-2.0µm), regular, gram-positive, non-
sporeforming rod-shaped bacterium with rounded ends that can be found as single
cells as well as in short chains. It can also appear spherical, and resembles
streptococci. *Listeria* spp. are catalase positive, oxidase negative, facultative
anaerobic or microaerophilic bacteria. They have peritrichous flagella, but the
motility differs markedly between species and depends on temperature and acidity of
the medium. *L. monocytogenes* is intercellular pathogen that uses host-produced
actin filaments for motility within a host cell. The bacteria propel themselves through
the cytoplasm, and then cross the cell wall into adjacent cells using a tail composed
of actin (44, 87).

*Listeria* spp. grow on most conventional media such as brain heart fusion,
tryptose broth, modified oxford agar (MOX) and MacConkey agar. When grown in
broth at 35-37°C, the media becomes turbid in 8-24 hours depending on species and
strain. On nutrient agar, the bacterial colonies appear mostly smooth, 0.2-0.8 mm in
diameter, and bluish-gray with blue-green hue after 24-48 hours at 35-37°C (43, 63 70). Listeria require the same nutrients for growth as most other gram-positive bacteria. Primary sources of nitrogen and carbon for Listeria are glutamine and glucose. In addition, these organisms require other amino acids like leucine, isoleucine, methionine, valine, cystine, cysteine, and at least four B vitamins: biotin, riboflavin, thiamine and thiotic acid as well as iron (85, 100, 124). Listeria spp. have several fermentation pathways and can produce adenosine triphosphate through a complete respiratory chain. All species are able to metabolize glucose to L (+) lactic acid by the Embden-Meyerhof pathway aerobically or anaerobically, but some strains can grow on lactose and maltose under aerobic conditions. Under anaerobic conditions Listeria can grow on pentoses and hexoses and can produce stable acid end products: lactate and acetate. In the presence of oxygen, these bacteria produce acetoin (19, 83, 94).

Growth characteristics of Listeria monocytogenes

Temperature

L. monocytogenes is psychrotrophic bacterium with optimum growth temperature between 30 and 37 °C, but growth can occur at temperatures as low as at -0.1 to -0.4°C (119). In 1994, Hudson et al. (50) reported growth of L. monocytogenes at -1.5 °C on modified atmosphere packaged sliced roast beef. It can survive freezing, and adapts to low temperatures by adjusting membrane fluidity and/or by the accumulation of compatible dissolved substances such as glycine,
betaine, proline, and carnitine (5, 9, 59). The growth range of *Listeria* spp. is broad, but its maximum growth temperature is at about 45°C (52).

**Acidity**

The influence of acidity on the growth and survival of *L. monocytogenes* varies by strain. It is dependent on pH, acid type, temperature, water activity, salt concentration and nutrient availability. The optimum pH for growth is 6-8, but it can grow in a pH range from 4.1 to 9.6 (52). Validated mathematical models have been developed for estimating growth behavior of *L. monocytogenes* through wide range of pH.

**Salt tolerance and required water activity**

*L. monocytogenes* is notably resistant to osmotic stress, and can survive at water activity below 0.93, though the growth range depends on interactions with acidity and temperature (31). It can grow in broth containing 10% (w/v) NaCl, but can survive in even higher salt concentrations (97). A major and well-characterized aspect of the salt tolerance of *L. monocytogenes* is the intracellular accumulation of the compatible solute- glycine betaine (102).

**Human listeriosis**

**Natural environment and transmission of *L. monocytogenes***

*Listeria monocytogenes* is a ubiquitous bacterium and often found in the soil, water, animal feeds and occasionally on plants. *L. monocytogenes* does not multiply
in soil but can be transmitted to the soil from human and animal feces, sewage sludge, silage and decaying vegetation. Welshimer (123) demonstrated that survival of *L. monocytogenes* in soil dependent upon type and moisture content. Lakes, rivers, and inshore marine waters can also be contaminated via sewage (23). Animals can be infected directly by contaminated water; however there have been no reported incidents of waterborne listeriosis in humans (45).

The gastrointestinal tract of healthy animal species can harbor *L. monocytogenes*. Since decaying vegetation is a natural environment for this microorganism, it is not surprising that the majority of grazing animals such as sheep, goats and cattle carry *L. monocytogenes*. The presence of the pathogen in feces of birds, pigs, rodents and other domestic animals is well documented. These animals can carry the bacteria for several months without symptoms of listeriosis and can contaminate foods of plant and animal origin such as fruits, vegetables, meats and dairy products (44). The incidence of *L. monocytogenes* shedding has been shown to be affected by the diet of animals (67) and some studies have shown increased excretion rates of the pathogen in stressed animals (33, 87). Humans, like many other mammals can be carriers of *L. monocytogenes* and it was found in the feces of 2 to 9% of healthy people (87).

Because of the widespread distribution of *L. monocytogenes* in animals and environment it can potentially contaminate equipment and a variety of foods. Raw foods, such as fresh meats, seafood, fruits, vegetables, unpasteurized milk, or foods made from unpasteurized milk, can harbor the pathogen and serves as a source of contamination. Processed foods such as soft cheeses, ice cream, deli meats and
frankfurters can become contaminated after processing (34). Because *L. monocytogenes* is an intracellular pathogen it can be found in interior muscle cores of animals as well. For example Johnson et al. (55, 56) demonstrated that the pathogen can be present in muscle tissues of Holstein cows at levels of approximately 140-280 CFU/g when animals were inoculated intravenously two days before slaughtering. Thus, inadequately processed meats could be a threat to the consumer, and the consumption of these and other contaminated products can result in human listeriosis.

**Epidemic foodborne listeriosis**

Immunocompromised people, pregnant women, newborns and children are particularly susceptible to invasive listeriosis (66). As mentioned, *L. monocytogenes* is an intracellular pathogen which can get into the blood stream through the wall of intestines, thereby allowing the infection to be spread anywhere in the body. The bacterium can invade macrophages, which makes it less susceptible to immune responses and less accessible by some antibiotics. It is also capable of intracellular multiplication, which causes death of the infected cell. Besides hematogenous spread, *L. monocytogenes* can be transmitted through neural routes to the brain and spinal cord causing neurological disorders (6, 26). Overall, the symptoms of the disease are variable depending on the susceptibility of the host but often include flu-like symptoms such as fever, headache, fatigue, nausea, vomiting, diarrhea, septicemia and meningitis. The symptoms of infection typically occur within 7-60 days after consuming contaminated food (120).
Infected pregnant women often experience nonspecific, mild, flu-like symptoms such as fever, muscle aches, and gastrointestinal problems (10). They can recover, but the infection can cross the placenta and infect the fetus causing miscarriage, premature labor, early rupture of the birth sac, and stillbirth. About 25% of infected babies die from this disease (101).

There are two types of neonatal listeriosis: early-onset disease and late-onset disease. Early-onset disease occurs in infants infected before birth and usually causes premature birth. Infected babies usually show sepsis, and sometimes granulomatosis infantriseptica. When a full-term infant shows the symptoms several weeks after birth, the disease is called late-onset listeriosis (118). The late onset disease includes meningitis about 93% of the time, but has a lower mortality rate than those with early-onset disease (71).

Outbreaks

The first confirmed outbreak of listeriosis in North America was documented in 1981 when 41 people became ill and 11 died after consuming contaminated coleslaw in Nova Scotia, Canada. The coleslaw had been prepared from cabbage that had been fertilized with raw sheep manure (96). Another large outbreak occurred in Boston, Massachusetts in 1983, transmitted by pasteurized milk which resulted in 49 cases and 11 deaths. The milk came from a farm where cows were known to be infected with listeriosis, however no defects in the pasteurization process were found. It was concluded that postprocessing contamination might have taken a place (35). The largest outbreak of listeriosis in North America occurred in
California in 1985 and apparently came from a Mexican-style soft cheese made from a mixture of inadequately pasteurized milk and raw milk. The outbreak resulted in 142 cases with 37% mortality rate of nonpregnant adults and 32% mortality rate of perinatals (64). Numerous outbreaks have involved RTE meats. In 1998, a multistate outbreak of *L. monocytogenes* infection occurred in North America associated with wieners from a Michigan meat processing plant, which caused the 101 cases with about 20% mortality (12). Another multistate outbreak of listeriosis in the United States occurred in 2000, causing 29 cases of disease, 4 deaths and three miscarriages (13). In 2002, 53 illnesses were caused by *L. monocytogenes* that resulted in 8 deaths and 3 miscarriages in the United States. Epidemiologic data indicated that contaminated precooked, sliceable turkey deli meat caused this outbreak as determined from food and environmental samples in two processing plants (14).

**Incidence and control of *L. monocytogenes* in RTE meats**

**L. monocytogenes prevalence in RTE meats**

Contaminated ready-to-eat meats pose a great threat because it is often consumed without further heating. The association of listeriosis outbreaks with unheated frankfurters has resulted in nonregulatory surveys to determine the incidence of *L. monocytogenes in* this product. Wang and Murina (121) examined 20 brands of retail wieners and found that 8 to 71% of these meat products were contaminated with *L. monocytogenes*. The liquid exudates harbored most of the pathogen (1-3 CFU/ml) indicating that contamination occurred after cooking,
because these products are removed from their packages after cooking and repackaged for sale, which can allow post-processing contamination from food handlers, equipment or air (51). Tiwari and Alrenrah (1990) reported a 17% incidence of *L. monocytogenes* in 38 samples of retail wiener and 67 samples of sliced deli meat in Alberta, Canada. Several surveys conducted in Europe revealed the presence of *Listeria* spp. (including *L. monocytogenes*) in a significant part of RTE meats in retail. A nine-year survey from a coordinated food sampling program in the United Kingdom, established in 1995, revealed that 1.7% of sliced RTE meats were contaminated with *Listeria* spp. which was the highest contamination rate among other RTE products (72). Numerous surveys in other countries have also indicated similar contamination rates (30).

**Use of Sodium Lactate (C₃H₅NaO₃)**

Sodium lactate (SL), the sodium salt of (L+) lactic acid, is found in a form of low-melting small crystals or a hydrophobic powder that has a mildly saline taste. It increases processing yield by increasing water holding capacity, but also increases flavor, shelf life and microbial safety of RTE meat products. The USDA-FSIS presently permits use of up to 4.8% (w/w) concentration of SL in RTE meats (36). The mechanism of bacterial inhibition of SL is not well understood, though there is some evidence that it can reduce water activity
enough to sufficiently inhibit bacteria (20). However, Weaver and Shelef (122) found that 4% (w/w) of SL in liver sausage did not lower the water activity enough to inhibit the growth of *L. monocytogenes*. The possible mechanism of action of SL on bacterial cell may arise from a lowering of cytoplasmic pH by undissociated lactic acid and possibly by metal chelation (98). Feedback inhibition and interference with proton transfer across the cell membrane also seems likely (104).

Shelef and Yong (99) reported that 5% (w/w) SL suppressed the growth of *L. monocytogenes* in tryptic soy broth. They also showed that SL inhibits *L. monocytogenes* growth in comminuted chicken or beef stored at 35, 20 or 5°C. Bedie et al. (8) showed that 3% of sodium lactate incorporated during preparation of the frankfurters had a bacteriostatic effect on *L. monocytogenes* in vacuum packaged frankfurters for more than 70 days, and 6% of SL had a bacteriocidal effect during 120 days of storage at 4°C. Samelis et al. (95) reported that 1.8% and 3% (w/w) SL used in frankfurters inhibited the growth of *L. monocytogenes* in a vacuum-packaged product stored at 4°C for 35 to 50 days. When used in combination with 0.25% sodium acetate, sodium diacetate, or glucono-δ-lactone, growth could be prevented for 120 days of storage. Mbandi and Shelef (69) also demonstrated a bacteriostatic effect by 2.5% SL when included in beef bologna with the growth of a five strain mixture of *L. monocytogenes* being suppressed for 60 days of storage at 5°C. Listeriocidal effects were also observed when 0.2% of SDA was included. Glass et al. (40) demonstrated that 3% SL or 1% SL combined with 0.1% SDA prevents listerial growth in wieners stored
anaerobically for 60 days at 4.5°C. The same study evaluated the use of 6% SL as a surface treatment and showed that growth of the bacteria was not delayed. Lu et al. (68) demonstrated ineffectiveness of SL as a surface treatment against *L. monocytogenes*, but found it to enhance the bactericidal effect of SD and potassium benzoate. Overall, most studies have shown SL be most effective against *L. monocytogenes* when included in RTE meats during formulation.

**Use of Lactic Acid (C₃H₆O₃) as a surface treatment**

![Lactic Acid Structure](image)

An important factor for controlling of *L. monocytogenes* growth and survival in foods is pH, where increased hydrogen ion concentration can limit bacterial growth (24). Lactic acid (pKa=3.79), a GRAS food additive, is produced naturally by lactic acid fermentation that is broadly used in the food industry to control spoilage and pathogenic bacteria. The mode of action of lactic acid (LA) is similar to other organic acids involving disruption of the proton motive force (27) and the induction of conformational changes of cell proteins, enzymes and nucleic acids (75).

Solutions of LA are used as dips or washes to remove or inhibit growth of spoilage and pathogenic microflora, including *L. monocytogenes* on meats during
storage under different conditions. Smulders and Woolthous (103) reported that
the spraying of veal carcasses with 1.0- 1.25% lactic acid decreased microbial
counts after vacuum packaged storage for 14 days at 2°C. Recent study by
Koutsoumanis et al. (61) showed that immersion of fresh beef, artificially
innoculated with five strains of *L. monocytogenes* in 2% LA at 55°C for 30s
decreased numbers by 1.43 log CFU/cm². One of the first studies to report LA to
control *L. monocytogenes* on RTE meats was done by Palumbo and Wiliams
(81) where the dipping of frankfurters inoculated with 5 strains of *L.
monocytogenes* in 2% solution of LA had bacteriocidal effect during 90 days of
storage at 5°C. In a recent multiple barrier study Barmapallia et al. (7)
demonstrated 0.7 log inhibition of 10 strain composite of *L. monocytogenes* on
artificially contaminated frankfurters by dipping in 2.5% solution of LA at 23°C for
two minutes. Bacterial growth was suppressed for over 40 days of storage at
10°C when 1.8% SL and 0.25% SDA were added to the formulation (7).

**Sodium Lauryl Sulfate CH₂(CH₂)10CH₂OSO₂Na**

Sodium lauryl sulfate (SLS) is an anionic surfactant with a structural balance
between a hydrophilic and hydrophobic group (25). SLS occurs as a small
crystals or as a white or pale yellow powder that is freely soluble in water giving
an opalescent solution, and partly soluble in alcohol. This GRAS chemical is used in the food industry for wetting, foaming and emulsifying purposes, as well as for sanitizing and antimicrobial purposes. The antimicrobial mode of action of this compound is not well understood but presumably, the primary mode of action involves general denaturation of proteins and enzymes and damaging cell membranes, causing changes in cell permeability. It has been found that anionic surfactants prevent bacterial attachment to the surface of the materials (25), and are generally potent antimicrobials against gram-positive and gram-negative microorganisms at acidic pH and at elevated temperatures. The antibacterial effect increases drastically at pH below 4 with an optimum pH range between 1.9 and 2.2 (25). In 1988 Hill and Ivey (48) patented a method for controlling *Salmonella* spp. on meat carcasses during processing. The method involves immersing the carcass for about 1 to 3 minutes in an aqueous solution of surfactants including SLS at pH below 4.0 and 45-60°C. They also suggested that this treatment can be used at lower temperatures for an appropriate amount of time. A similar acid-synergist approach was tested against various bacterial strains in a study of Restaino et al. (90) where the antimicrobial properties of a buffered organic acid anionic surfactant (BOAAS) containing SLS, citric acid, and chelating agent (EDTA) was compared with six other conventional sanitizers. This antimicrobial treatment displayed superior activity compared to other sanitizers against *Pseudomonas aeruginosa, Salmonella Typhymurium, Staphylococcus aureus* and *L. monocytogenes*. The initial numbers of the pathogens were reduced by more than 5 log -cycles on the surface of artificially
contaminated Formica countertop after 30-sec exposure to a 0.6% solution of BOAAS. Tamblyn and Conner (109) used an aqueous solution of 125 ppm of SLS and 0.5% of LA against Salmonella Typhimurium broiler chicken skin surfaces. They demonstrated 0.82-1.54 log CFU/skin reduction of initial numbers of loosely and firmly attached bacterial cells after exposure to the solution for 2 minutes at 50°C. Immersion of the skins in the solution for 60 minutes at 0°C reduced loosely and firmly attached bacterial cells by 0.52 and 1.30 log CFU/skin respectively. Takeuchi and Frank (110) evaluated decontamination of lettuce artificially contaminated with E. coli O157:H7 using a prototype fruit and vegetable alkaline (pH=11.1) washing solution containing SLS, baking soda, ethyl alcohol, oleic acid, citric acid and distilled grapefruit juice. At 22°C, they demonstrated the solution lower counts by 0.7-1.1 log CFU/cm² when rinsed for 3 minutes. A recent study by Raiden et al. (86) demonstrated that a solution of 0.1% SLS did not possess bacteriocidal activity against a five-strain cocktail of Salmonella spp. during incubation for 32 hours at 22 and 40°C. This study implies that SLS is ineffective as an antimicrobial at neutral or high pH and a role SLS in alkaline detergent solutions when used as a surface rinse is linked to the detachment of cells from the surface rather than their destruction of viable cells.
Irradiation of foods

Brief history

In 1895 a German physicist, Wilhelm Roentgen, discovered a form of radiation that could knock electrons loose from atoms when passing through materials that absorb visible light. He called it X-radiation because of its unknown nature. Later, in 1896, French physicist Becquerel discovered radioactivity through his investigations of uranium and other substances; he was awarded the 1903 Nobel Prize for physics. Use of ionizing radiation to destroy microorganisms in food was first patented by Appleby and Banks in England in 1905 (22). In 1921 a US patent was issued to Schwartz for the use of irradiation to eliminate Trichina spiralis in meats. Ionizing radiation was known to kill bacteria, but active study of food irradiation started in the late 1940's, when the technology of production radioisotopes and electrons became available. In 1953, the US government formed National Food Irradiation Program which was largely sponsored by the U.S. Army and the Atomic Energy Commission the numerous research projects involving the irradiation of foods. Early studies demonstrated that irradiation could be applied to different foods without making them radioactive, though accumulation of radiolytic products was observed. By the time the United States Drug Administration inherited the U.S. Army's food irradiation program in 1980, the Food and Drug Administration defined sources of radiation intended for use in food processing as a new food additive. The FDA also approved irradiation for a number of food products and packaging materials (57). Currently, the Federal Government allows the irradiation of refrigerated and frozen uncooked
meat, meat byproducts, and a few other meat food products, to reduce the levels of foodborne pathogens and extend shelf life. Irradiation is also approved for some other uses in the food industry including: elimination of insects from grains and flour, inhibition of sprouting in potatoes, and *Salmonella* spp. control in fresh shell eggs. Use of irradiation to control pathogens in RTE processed meats such as deli meats and frankfurters, which can become contaminated with *L. monocytogenes*, is under consideration (38).

**Nature of Irradiation**

Radiation may be defined as emission and propagation of energy in the form of waves or particles through space or a material. The energy in electromagnetic spectrum exists as waves, which are differentiated by the wavelength, frequency, and penetrating power of radiation. Ionizing radiation includes x-rays, beta rays and gamma rays which exist as invisible short waves that can penetrate foods and cause changes in atomic structure of their molecules. Ionizing radiation can destroy pathogens without changing the temperature and so it is frequently referred to as a cold pasteurization. In contrast with other waves, x-rays, gamma and beta rays can eject electrons from an outer shell of an atom by energy transfer from photons. These very energetic waves, having wavelength of 2000 Å, can be used on foods without inducing radioactivity. X-rays, beta rays, and gamma rays also have differing penetration power. Beta rays possess weak penetration capacity and have little potential for applications in food industry. X-rays have higher penetration capacity, but its application in the food industry is limited because of the difficulty in
focusing them on a food product. Gamma rays have excellent penetration power; they can penetrate material with thicknesses of about 40 cm and are more cost effective for food preservation than other ionizing radiation waves (88, 52, 73).

The sources of irradiation permitted by Federal Government are radioisotopes such as $^{60}\text{Co}$ and $^{137}\text{Cs}$, electron beams from linear accelerators and sub-atomic particle accelerators (16). Cobalt-60 is an artificially produced radioactive isotope of cobalt formed by placing non-radioactive cobalt-59 pellets in a nuclear reactor for neutron bombardment. Cesium-137 is produced when other radioactive materials, such as uranium and plutonium, absorb neutrons and undergo fission. Both $^{60}\text{Co}$ and $^{137}\text{Cs}$ constantly emit highly penetrating gamma rays, which can be used for food preservation. Cobalt-60 is preferable for the food industry not only because the technology for its production is highly developed but also its penetration capacity is superior to $^{137}\text{Cs}$. However, it has a short half-life (5.2 years for $^{60}\text{Co}$, as opposed to 30 years for $^{137}\text{Cs}$). This means that part of the radioisotope constantly needs to be replaced to maintain the initial power. Electron-beam accelerators became a better alternative for generating irradiation because the price for cobalt-60 has increased in recent years and there exist a public concern about radioactive materials (107). Also, electron-beam machines have other advantages over use of radioisotopes including: high efficiency, simplicity of programming and control of irradiation dose and direction of electron flow, and ease of turning them off (60).

The most important factor in the irradiation of foods is the dose applied to the foods. The absorbed dose, once measured in rads is now measured in grays (Gy), (1Gy=100 rads=1 joule/kg; 1000Gy=1kGy). Also, the absorbed dose can be
expressed in electron volts (eV), i.e. energy gained by an electron moving through 1 V (52, 88, 73).

Depending on the microbiological objectives, irradiation treatment of foods can be classified as radappertization, radicidation and radurisation. Radappertisation (30-40 kGy), is the analog of “commercial sterility” in the canning industry. Radicidation, (2.5-10 kGy) aimed at destroying viable, non-sporeforming pathogens and is the analog of pasteurization. The objective of radurization (0.75 to 2.5 kGy) is to reduce the population of vegetative spoilage bacteria. Although radurization can be used on a variety of foods, the efficacy of this method for improving microbial quality may be restrained by possible survival pathogenic and gram-positive bacteria (41, 52, 73).

The effects of ionizing radiation

As ionizing radiation travel through organic materials, such as food and microorganisms, it interacts with atoms, transferring some of its energy. The energy lost by the ionizing radiation is absorbed by the material through which the radiation is traveling. Absorbed energy can cause chemical changes in the organic matter. During irradiation, photons or electrons collide with atoms in a molecule and expel the electron from an atom leaving the molecule positively charged. This ionizing radiation changes neutral atoms or molecules into electrically charged atoms or molecules, or ions. A molecule containing at least one unpaired electron is called a
free radical. When exposed to irradiation, the molecules of the organic material can form free radicals and expel electrons:

\[ M \xrightarrow{\text{irradiation}} \cdot M^+ + e^- \]

Free radicals are usually highly reactive and take part in various chemical reactions, and expelled electrons can dissipate electrons from other atoms and molecules. Passage of gamma rays through molecules and atoms does not necessarily strip the electrons from them, but induces them to become "excited", which is called excitation.

\[ M \xrightarrow{\text{irradiation}} M^+ \]

Excited molecules keep the received energy for about $10^{-8}$ seconds and lose it by transferring it to other molecules, internal conversion to heat, or through a variety of chemical reactions (46).

The energy impaired by ionizing radiation is non specific and can affect normal functions of biological systems. In bacterial cells, excitation or ionization affects DNA as well as cell membranes and other biological structures (88, 107).

**Direct and indirect action**

Ionizing radiation affects microorganisms directly by damaging microbial DNA (52). Depending on the orientation of the DNA molecule, single- or double-strand damage can occur. Microorganisms are capable of repairing some single-strand
lesions but may not survive large numbers of damaged sites. Double-strand breakage occurs when the photon or electron hits nearby areas on both DNA strands, but occurs 10 to 20 times less frequently than single strand breakage because the orientation of DNA molecules in relation to the source of irradiation does not often favor such damage (21, 76). The damage cleaves the DNA molecule in half and often causes bacterial death.

Ionizing radiation also may have indirect effects on organic molecules through water radiolysis, since about 80% of every cell is composed of water. The water molecule becomes ionized and forms an ion radical, \( \bullet H_2O^+ \) and a free electron, as a consequence of the interaction with a photon:

\[
H_2O \quad \text{irradiation} \quad \bullet H_2O^+ + e^-
\]

Further, a chain of reactions occurs, yielding secondary radicals and their products including very reactive hydroxyl and hydrogen radicals, hydrogen, hydrogen peroxide and solvated water (80). These products can oxidize or reduce molecules in a cell and thus, disrupt the cytoplasmic membrane, enzymes, and proteins, as well as damage bacterial DNA, which leads to cell death. The effect on DNA by free radicals is the same as irradiation direct effect. Because free radicals can react with nucleic acids they disrupt bonds between and within DNA strands of bacterium. The injury created by free radicals, together with the hydroxyl radical is irreparable if molecular oxygen is present and results in cell death (80, 116, 117). Reaction of free radicals with each other and with other molecules eventually stabilizes radiolytic products. These processes are transitory in nature and vanish in \( 10^{10} \text{-} 10^{11} \text{ m}^{-1} \text{s}^{-1} \) (21). Resistance of living organisms to ionizing radiation depends on radiation dose,
types and numbers of microorganism, composition of surrounding medium, and temperature and atmospheric conditions. Also, bacterial cells in stationary or lag phase of growth are more resistant to irradiation than cells in exponential phase (32, 52, 73, 88).

**Factors that contribute to microbial resistance to irradiation**

**Irradiation dose**

Destruction of bacteria by irradiation is dose-dependent, and like heat inactivation, can be expressed as a D-value, the dose required to reduce number of bacteria 10-fold or 90% under stated conditions (77). The resistance of the bacterial population to ionizing radiation can be represented by plotting of the logarithm of the survivors versus irradiation dose. Ideally, this generates a straight line where D-value equals the absolute value of the inverse of the slope. It is worth mentioning that ionizing radiation used at doses permitted for food use does not destroy toxins produced by microorganisms (21, 88).

**Microbial factors**

Ability to repair irradiation damage varies greatly between microorganisms, and it increases with complexity and size of the organism. Thus viruses are more resistant than bacterial cells, as the latter contain more genetic information in their DNA; yeasts and molds are even less resistant. Bacterial sensitivity to irradiation varies between strain and species, but in general, gram- negatives are more sensitive than gram positives, and rod shaped bacteria are more sensitive than
cocci. Non-sporeformers are less resistant to irradiation then sporeformers. Spores are more resistant to irradiation mainly because of low water content in a spore protoplast; however radiation resistance disappears rapidly upon germination. Also, effectiveness of a given irradiation dose can be reduced by larger number of microorganisms.

**Food composition**

The composition of food, its water activity, and the presence of other antimicrobials can significantly alter radiation sensitivity of microorganisms. Bacteria present in dehydrated foods or foods with high salt or sugar content are more resistant to ionizing radiation because less water is available for formation of radiolytic products, decreasing the extent of indirect effect. Food components, primarily proteins and natural antioxidants, (i.e. vitamin C and vitamin E) can neutralize free radicals (73), and thus reduce the effect of irradiation. Additionally, Steccini et al. (106) reported that carnosine, dipeptide of beta alanine and histidine have antioxidant properties and increased the irradiation resistance of *Aeromonas hydrophila* in minced turkey meat. The protective ability of proteins was reported by Midura and coworkers in 1965 (74). They reported a 0.23 kGy D-value for *Clostridium perfringens* in phosphate buffer, while the D-value in cooked meat broth was 3.0 kGy. On contrary, lipids can contribute to the injury of microorganisms during irradiation due to formation of free fatty acids, carbonyl compounds, hydrogen peroxide and hydroperoxides, but the injury can be diminished by the presence of proteins and other protective components in a food system (73). In general, a
complex media protects microorganisms from irradiation injuries by competing for radiolytic products generated by the process (32).

**Temperature**

The temperature of a food at the time of irradiation also influences bacterial resistance to irradiation. The lethal effect of ionizing radiation on vegetative cells is synergistically enhanced at higher temperature perhaps because bacterial repair systems are damaged by higher temperatures, especially above 45°C. Freezing decreases water activity and consequently significantly increases the resistance of bacterial cells to irradiation. Another reason for higher resistance of frozen vegetative cells is slower diffusion rate of radiolytic products and hence, slower rate of chemical reactions in the cell, which diminishes indirect DNA damage. Numerous studies have indicated the essential role of irradiation temperature in destruction of viable bacterial cells (32, 73). Thayer and Boyd (112) irradiated lean beef meat inoculated with *L. monocytogenes* at several temperatures from -60 to +15 °C and found that a sudden increase in resistance occurred at -5 degree C. They used this research to develop a predictive model for the response of *L. monocytogenes* to gamma radiation within a given temperature range. A recent study of Niemira et al. (79) confirmed the effect of the temperature on the radiation resistance of *L. monocytogenes* and showed that the D-values increased significantly with decreasing temperature in frozen vegetables.
Gaseous composition

The lethal effects of ionizing radiation increases with increased atmospheric oxygen concentrations (29, 49, 114). Sensitivity to irradiation decreased two to four times in humid aerobic conditions, but in aerobic conditions with low humidity the sensitivity decreased eight to seventeen times (32). Thailer and Boyd (111) showed that irradiation is more lethal to *L. monocytogenes* in turkey meat packaged aerobically than in the meat packed anaerobically or in a modified atmosphere. Other studies have indicated no significant difference between irradiated vacuum packaged ground beef and the ground beef packed aerobically for total bacterial counts and *E. coli* O157:H7 (39, 65). Microbial resistance to irradiation is also affected by the composition of modified atmosphere used in packaging. *Lactobacillus* spp. in ground beef were found to be more resistant to irradiation when packaged in nitrogen as opposed to the beef packaged in carbon dioxide (47). Grant and Patterson (1991) demonstrated that minced pork irradiated at 1.75 kGy and packaged in containers with 25% CO$_2$:75N$_2$, had significantly higher D-values for two strains of *L. monocytogenes* than samples irradiated in an unmodified atmosphere.

Radiosensitivity of *Listeria monocytogenes* in meat products

There are numerous reports on the effects of gamma radiation on *L. monocytogenes* in meats. D-values of 0.4 to 1.0 kGy have been reported depending on serotype, growth phase, irradiation temperature, water activity, salt and oxygen concentration, and atmospheric pressure. As mentioned, D-values for *L. monocytogenes* in meat products is relatively high due to high protein content, and
the antioxidative properties of some peptides and vitamins in the products (82, 106, 111). Patterson (82) reported D-values of 0.42-0.55 kGy for *L. monocytogenes* in poultry meat depending on strain and poultry medium. Recently, Romero et al. (93) irradiated ground turkey meat with 0.5-2.0 kGy and reported D-values of 0.56 to 0.59 kGy for a five strain mixture of *L. monocytogenes*. El-Shenawy et al. (28) showed D-values of 0.51 to 1.0 kGy for various strains of the pathogen in irradiated ground beef. Thayer and Boyd (113) presented evidence that resistance of *L. monocytogenes* to ionizing radiation in different meats is the same if irradiation and cultural conditions are identical. In their study they irradiated (0.25 to 2.0 kGy) inoculated, and vacuum packed beef, lamb, pork, and turkey breast and leg meat; the D-values for four strains of *L. monocytogenes* were 0.47 kGy in all tested types of meat. In a separate study, Thayer and coworkers (111) showed that the D-value for destroying four isolates of *L. monocytogenes* on raw turkey nuggets after irradiation with 0.5 to 3.0 kGy averaged 0.56± 0.03 kGy whereas D-values for cooked nuggets averaged 0.69± 0.03 kGy. They concluded that cooking produced substance(s) that protected the bacterial cells from irradiation.

The radiosensitivity of *L. monocytogenes* varies in RTE meats as well. Clardy et al. (15) irradiated (3.5 to 4.0 kGy) ham and cheese sandwiches, inoculated with $10^7$ CFU of *L. monocytogenes*. They found that the D-values ranged from 0.71 to 0.81 kGy. Somers et al. (105) revealed that salts of organic acids can decrease resistance of *L. monocytogenes* to irradiation. In this study D-values for *L. monocytogenes* were 0.46 to 0.53 kGy in bologna containing 0.07% to 0.15% sodium diacetate and 1.0 to 2.0% sodium lactate were, whereas for samples
formulated without these salts, the D-value averaged 0.56 kGy. Recent studies of Foong (37) reported D-values of 0.42 to 0.44 kGy for a five-strain cocktail of *L. monocytogenes* in irradiated (2.0 to 4.0 kGy), vacuum-packaged RTE meats. These included frankfurters, ham, roast beef, bologna, smoked turkey with lactate, and smoked turkey without lactate. Zhu et al. (125) reported D-values of 0.48 to 0.52 kGy for *L. monocytogenes* in irradiated (2 to 5 kGy) turkey ham containing 2% sodium lactate, 0.1% sodium diacetate, 0.1% potassium benzoate or combinations of these antimicrobials. Although, radurization can eliminate *L. monocytogenes* from meat products, numerous studies have shown that, in the absence of multiple treatments, low numbers of the pathogen may survive and subsequently proliferate during refrigerated storage (18).

**Quality changes of irradiated meat products**

**Color**

Color is the most important attribute of RTE meat affecting consumer buying decisions and influencing their quality perception of the product. Fresh and cured meat color depends on muscle type, types of pigment and concentration, as well as the presence and nature of other ingredients and preservatives in the processed products. Irradiation may affect the color of the meat products by the interaction of radiolytic products with heme pigments in meat. This is dependent on pigment content of the meat, irradiation dose, dose rate, packaging environment, and the atmosphere and temperature during the storage (3). Results of studies reporting the effect of low dose irradiation on the color have not been consistent because of the
variety of affecting factors. Nanke et al. (78) irradiated fresh vacuum-packaged pork, beef, and turkey at various irradiation dose levels. They concluded that the pork and turkey develop a pink color, at doses of 4.5 kGy to 7.5 kGy and 1.5 kGy to 3.0 kGy respectively, whereas the beef developed greater brownness at these doses. Fu et al. (39) reported that irradiation of beef steaks with 1.5 kGy caused no changes in redness, lightness and yellowness. Romero et al. (93) revealed that irradiation of ground turkey meat with 0.5 kGy to 2.0 kGy cause a dose-dependent elevation of Hunter a-values and decreased L-values, while the b-value were unaffected.

Changes in the color of RTE meats have been studied less intensively than that of raw meats. Jo et al. (53) reported that irradiation with 4.5 kGy increased Hunter a-values while b-values were reduced in vacuum packaged pork sausage. In aerobically-packaged samples a-values decreased but b-values did not change. Lightness was not affected in both aerobically and vacuum packaged pork sausage samples. In a separate study, Jo et al. (54) irradiated (2.5 to 4.5 kGy) vacuum-packaged and aerobically-packaged pork sausage and confirmed a dose-dependent increase of the redness in vacuum-packed samples, while Hunter a-values in aerobically-packaged samples decreased. The Hunter b-values in this study were reduced by irradiation, but lightness was not affected regardless of type of the packaging. Similar results were reported by Byun et al. (11), who demonstrated elevating of redness and a subsequent decrease in lightness with no changes in yellowness of vacuum-packaged irradiated (5.0 kGy) pork lion. In a more recent study, Ahn et al. (4) evaluated the effect of irradiation at 5.0 and 10.0 kGy of
modified atmosphere packaged, nitrite cured cooked pork sausage. The study concluded that irradiation significantly reduced redness in the nitrite-cured meat product regardless of modified atmosphere used in the packaging.

**Irradiation odor**

Odor is also a very important organoleptic property of meat products. Irradiation odor develops in meat after irradiation due to radiolytic products causing oxidation of myoglobin and fat, which leads to rancidity and other off-odors (77). Jo and Ahn (54) suggested that off-odor of irradiated meats were due to radiolytic degradation of amino acids, primarily sulfur amino acids. The radiolytic sulfur-volatiles have much greater effect on overall off-odor of irradiated meat than lipid oxidation-dependent volatiles like ketones and aldehydes (2). Odor characteristics are dose dependent and have been described as being barbecued corn-like, metallic, sulfide, wet grain, burnt, and wet dog (1, 89). Sudarmadji and Urban (108) revealed that the threshold for irradiation odor formation depends on type of species and ranged from 1.5 kGy for turkey meat to 6.25 kGy for lamb. Factors contributing to the rate of primary and secondary reactions of free radicals are believed to affect off-odor formation in irradiated meat. In contrast, when meat products are irradiated under vacuum conditions, most of the sulfur volatiles are retained in meat whereas these compounds disappear if the product was irradiated and stored in presence of oxygen (2). Processed meats tend to develop less irradiation odor due to antioxidative activity of spices. In cured meats the oxidative changes can be
diminished by sodium nitrite (58). The mechanisms and chemistry of volatile production are out of scope of this review.
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CHAPTER 3. EFFICACY OF LACTIC ACID ALONE OR COMBINED WITH SODIUM LAURYL SULFATE FOR CONTROL OF _Listeria monocytogenes_ IN VACUUM PACKAGED FRANKFURTERS MADE WITH OR WITHOUT SODIUM LACTATE

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**Abstract**

The inhibition of _Listeria monocytogenes_ by lactic acid (LA) and sodium lauryl sulfate (SLS) was evaluated in frankfurters containing 64% beef and 36% pork and made with or without 2% (w/w) sodium lactate (SL). The frankfurters were surface treated by dipping for either 1 or 3 minutes in solutions containing all combinations of 0 and 5% (v/v) LA and 0.0, 0.5 and 1% (w/v) SLS. The treated product was surface inoculated with \(10^7\) CFU of a five-strain mixture of _L. monocytogenes_ per link, vacuum packaged and stored for at 4.0° C for 90 days. Dipping in 5% LA or 1% SLS alone reduced initial numbers of the pathogen by 0.4 to 0.7 log. In contrast, the combination of 5% LA and 1% SLS reduced initial numbers of the pathogens by 3.8 log CFU/link. Dipping time did not have an immediate inhibitory effect on the initial population of the pathogen but there was some residual bacteriostatic effect when samples with SL were dipped for 3 minutes in LA (5%) + SLS (1% or 0.5 %). Number
of survivors did not increase on frankfurters that contained SL. The Hunter L-, a- and b-values were not affected by the dipping solutions or by 2% SL. The pH of the frankfurters was not changed by SL, but dipping in solutions that contained 5% LA decreased pH of the product (P<0.05). Based on these results the use of LA (5%) +SLS (1%) for surface treatment of frankfurters formulated with SL (2%) has good potential for control of *L. monocytogenes* in these RTE meat products; however sensory evaluation studies are needed to evaluate the influence of the treatments on quality changes.

**Introduction**

*Listeria monocytogenes* is a gram-positive psychrotrophic foodborne pathogen that is widely distributed in nature and its presence in foods is a major concern for food regulatory agencies, food processors, and consumers (25, 48). It causes approximately 2,500 cases of food poisoning per year in the United States with a case fatality rate of 20-30% (8) and its long incubation period make the illness difficult to trace (21, 40).

About 20% of human listeriosis arises from the consumption of contaminated and improperly heated frankfurters or chicken and thus these ready-to-eat (RTE) meats pose the greatest risk to public health (16, 20, 25). *L. monocytogenes* does not survive the cooking of frankfurters (55), but contamination can occur during peeling and before packaging. RTE meats have the pH, water activity, and nutrients to support moderate to high growth of the pathogen which can grow at refrigeration temperature (23). Therefore, it is not surprising that these meat
products have been implicated in outbreaks of listeriosis. In 1998, an outbreak associated with wieners from a Michigan meat processing plant resulted in 101 cases of listeriosis and 20 deaths (7). In 2002, two separate listeriosis outbreaks were linked to sliced RTE turkey meat products (8, 12).

Based on the characteristics of *L. monocytogenes* and the severity of listeriosis and outbreaks, the U.S. Department of Agriculture, Food Safety and Inspection Service, and the Food and Drug Administration established a “zero tolerance” rule for *L. monocytogenes* in ready-to-eat meats (13, 19, 41). Also, an interim final rule was issued in June 2003, encouraging all plants to implement additional and more efficient measures for control of *L. monocytogenes* (22, 53).

Numerous studies have investigated the effect of lactic acid and sodium lactate applied as dipping solutions or as formulation ingredients on growth of *L. monocytogenes* on RTE meats (2, 9, 28, 30, 31, 32). Sodium lactate can suppress the growth of *L. monocytogenes* at concentrations that do not affect quality of comminuted sausages when it is added during formulation of this meat product (10). Lactic acid (LA) suppresses the growth of *L. monocytogenes* on surface of the frankfurters (32) by lowering pH and acidifying the cytoplasm. When used as a dip, 2.5% LA (2 min dip) reduced initial populations of *L. monocytogenes* on frankfurters by 0.7-2.1 log-cycles (2) and listeriocidal activity of 3.5% LA was shown (31).

The surfactant, sodium lauryl sulfate (SLS) is a generally regarded as safe (GRAS) food additive (10 ppm-0.5%) that is used in animal fats, vegetable oils,
fruit juices and beverages, gelatin, marshmallows, egg whites (11). SLS is also thought to damage the cell membranes and denature proteins of microorganisms, where its activity is enhanced below pH 4.0 and at elevated temperatures (17). Tamblyn and Conner (52) demonstrated that 125 ppm of SLS in combination with 0.5% LA reduced initial counts of *Salmonella* Typhimurium attached to broiler skins by 1.3 log-cycles. A patent was issued to Hill and Ivey in (24) for a method of controlling *Salmonella* spp. on poultry carcasses by using these two compounds. Restaino et al. (39) demonstrated that a 0.6% solution of SLS, citric acid and EDTA inactivated > 5 logs of *L. monocytogenes* on the surface of Formica countertop after a 30-sec exposure. However, Raiden et al. (37) found that *Salmonella* and *Shigella* spp. can survive in 0.1% SLS. They further reported that 0.1% solution of SLS did not enhance the removal of the pathogens from the surface of fresh produce (38). This can be explained by ineffectiveness of SLS as antimicrobial agent when used alone at low concentrations and at pH close to neutral.

Preliminary studies in our laboratory indicated that the antilisterial effectiveness of lactic acid increased drastically when this organic acid was combined with sodium lauryl sulfate. Our observations are consistent with the concept of acid anionic surfactant technology in which anionic surfactants exhibit increased antimicrobial activity in an acidic environment (17). To our knowledge, there is no published research on the combined effect of sodium lauryl sulfate and lactic acid against *L. monocytogenes* on frankfurters. The objective of this study was to evaluate the effectiveness of lactic acid in combination with sodium
lauryl sulfate as a dipping solution for destroying *L. monocytogenes* on the surface of frankfurters and to evaluate the effect of these treatments on the pH and color of the product.

**Materials and methods**

**Experimental design**

Six dipping solutions including the control, two dipping times (1 and 3 min) seven storage times (1, 14, 28, 42, 56, 70, and 90 days) at 4°C, and two types of frankfurters (0% SL and 2% SL) were used in present study. Three replications of the treatment were carried out. The experimental unit for the dipping solutions and dipping time treatments was two frankfurters. A randomized complete-block full factorial experimental design was used.

**Preparation of frankfurters**

Frankfurters were prepared with pork fat trimmings (40/60 lean/fat ratio) and lean beef trimmings (80/20 lean/fat ratio) at Iowa State University Meat Laboratory. The meat was ground through a 0.79-cm grinder plate, and divided into two batches. Sodium lactate, as a powder (SL; Purac Inc., Lincolnshire, IL) was added in one batch along with salt, sodium erythorbate, sodium nitrite, seasoning and water with ice during emulsification in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA.). Another formulation batch served as a control, and was prepared the same way as the first one using the same ingredients without SL. The meat batter was extruded through a meat stuffer (*Risco*® Model RS 4003-
into 22-mm peelable cellulose casings (Devro Teepack™ Wiene-Pack® Coastal corrugated Inc., N. Charleston, S.C., U.S.A.). The sausage was linked at 14.0 cm length by 2.2 cm in diameter using a poly-clip system (GmbH and Co., KG, Frankfurt, Germany).

The linked product was hung on racks and cooked for 90 minutes using the conventional cooking-smoking cycle in a humidity-controlled smoke house (Alkar, DEC Intl. Inc., Lodi, Wis., U.S.A.) to an internal temperature of 71.1° C. Natural smoke (hardwood sawdust; Frantz Co., Milwaukee, WI) was applied during the cooking cycle. At the end of the cycle, frankfurters were showered with cold water, and then kept in a walk-in refrigerator at 4.0°C for 18-19 hours. The following day frankfurters were peeled, (Peeler Townsend 2600, Des Moines, IA) sealed in vacuum bags, then stored at -20°C in a walk-in freezer until used.

**Treatment of frankfurters**

Frozen frankfurters for each replicate were transferred to a walk-in refrigerator and thawed overnight at 4°C. After thawing, frankfurters were aseptically removed from the bulk package and placed into a sanitized aluminum basket (28 samples per basket) and immersed in freshly prepared solution (3 liters) of 5% (vol/vol) lactic acid (LA; Birko Co. Denver, CO.), 0.5% (wt/vol) sodium lauryl sulfate(SLS; Fisher Scientific, Fair Law, NJ), 1% SLS, 0.5% SLS + 5% LA, 1% SLS+ 5% LA, or deionized water (control) for 1 or 3 min at room temperature (23±1°C). After immersion in the treatment solution for 1 or 3 min, each basket of frankfurters was removed and placed over a plastic container to allow the samples to
drain for about 2 minutes. Each frankfurter was placed in a separate vacuum package bag (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission=0.5-0.6g at 100°F, 100% RH/100 sq.in./24h; oxygen transmission rate=36 CC at 40°F/m²/24h/0%RH) prior to inoculation.

**Preparation of inocula**

Five strains of *L. monocytogenes* were used: H7962 serotype 4b, H7969 serotype 4b, H7762 serotype 4b, Scott A/NADC 2045 serotype 4b, and H7764 serotype 1/2 a. Except for *L. monocytogenes* Scott A, which was obtained from the National Animal Disease Center, Agricultural research Service, U.S. Department of Agriculture, Ames, Iowa, all strains were isolates from the 1998-1999 Bil Mar Food Outbreak (CDC, Atlanta, GA). Cultures were maintained as frozen(-70°C) stocks in brain heart infusion broth (Difco Becton Dickinson, Sparks, MD.) with 10% glycerol until used to prepare working cultures. Working cultures of each *L. monocytogenes* strain were prepared by transferring a loopful of stock culture into 10 ml of tryptic soy broth (Difco) supplemented with 0.6% yeast extract (Difco; TSBYE) followed by incubation at 35°C for 18 hours. Prior to each replication of the experiment, two consecutive 18-h transfers of each *L. monocytogenes* strain were prepared in TSBYE at 35°C. A five-strain cocktail was prepared by transferring 6 ml of each culture into a sterilized 30-ml centrifuge tube. Cells were harvested by centrifugation (10,000×g, 10 min, 4°C) and the supernatant was discarded. Pelleted cells were suspended in 30 ml of 0.1% peptone water and washed by centrifugation. This procedure was repeated twice to obtain washed cells. The washed cell suspension
was diluted 10-fold in order to obtain $10^8$ CFU per ml, which served as the inoculum. Cell population in the inoculum was determined by plating on tryptic soy agar (TSA; Difco) supplemented with 0.6 % yeast extract (TSAYE) plates and incubation at 35°C for 24 h.

**Inoculation of frankfurters**

Each frankfurter was spot-inoculated with 0.1 ml of the five-strain cocktail of *L. monocytogenes* to give initial population of $10^7$ CFU/link. Inoculated frankfurters were then manually massaged from outside of the bag for 10 sec. to spread the inoculum over the surfaces. Bags of inoculated frankfurters were vacuum packaged at 95 kPa with MultivacA 300/51 vacuum packaging machine (Multivac Sepp Haggenmüller, Gmbt & Co., Wolfertschwenden, Germany) and stored at 4°C for 90 days in a walk-in refrigerator.

**Measurement of pH**

The pH of frankfurters was determined by procedure described by Sebranek (45). A 10-gram sample of each non-innoculated frankfurter with 90 ml of distilled 0.1% peptone water was pummeled using a Seward Stomacher 400 Lab-blender (Seward Ltd., London, England) for one minute at medium speed. The pH value of frankfurter slurry was measured using an Orion Model 525 pH meter (Orion Research Inc., Boston. MA) fitted with a glass electrode. Measurements of pH were conducted on days 1, 14, 42, 70 and 90 during the storage of samples.
Color measurement

Non-inoculated frankfurters were evaluated at days 1, 14, 42, 70, 90 to determine changes in color. A Hunter Lab Scan Colorimeter (Hunter Associated Labs. Inc., Reston, VA) was calibrated against black and white reference tiles covered with the same packaging materials as that used for samples. Samples were measured at three different locations on the surface. The results were expressed as Hunter L (lightness), a (redness), b (yellowness) values. Area view and port size were 0.25 and 0.40 inch, respectively.

Microbiological analysis

Vacuum packaged frankfurters were opened aseptically and 20 ml of 0.1% sterile peptone water (Difco) were added to each package. Samples were vigorously massaged by hand from outside of the package for 30 seconds. Ten-fold serial dilutions of the wash solution were prepared in 0.1% of peptone water. Aliquots (0.1 ml) of appropriate dilutions were surface-plated onto modified Oxford agar (MOX; Difco). All inoculated agar plates were incubated at 35°C for 48 hours and typical colonies of L. monocytogenes were counted after incubation and expressed as log CFU per frankfurter.

Statistical analysis

Three complete replicates of the experiment were conducted each with two samples per treatment. Analysis of variance was used to assess the significant
differences among the mean values. Tukey’s honestly significant difference test was used to determine whether there was a significant difference in microbial reductions, pH and Hunter color $L_-$, $a$- and $b$-values. Differences were considered statistically significant when the associated $P$-value was less than 0.05 (45).

**Results and discussion**

The effect of SL (as a part of formulation), LA, and SLS (as a surface treatment) alone or in factorial combinations on the initial population of *L. monocytogenes* on frankfurters was evaluated. Preliminary trials indicated that almost complete recovery of the inoculum could be achieved using the methodology, described in the present study. As shown in Figure 1, dipping frankfurters in distilled water, 5% LA alone or 0.5% SLS alone before the inoculation did not have an antimicrobial effect ($P>0.05$), whereas 1% SLS alone reduced the initial *L. monocytogenes* population by 0.7 log CFU/link. This finding is not consistent with other studies, which indicated a reduction of *L. monocytogenes* on the surface of frankfurters by immersing in lactic acid (2, 31, 32). This discrepancy may be attributed to inoculation prior to dipping, which was done in those studies. This increased inactivation of *L. monocytogenes* on inoculated frankfurters that are dipped in antimicrobial solution may be attributed to both physical removal of loosely attached cells as well as complete immersion of the attached cells in the treatment solution. Our method of inoculating the frankfurters after dipping them in the antimicrobial solutions takes into consideration that frankfurters removed from treatment solution are still vulnerable to contamination with *L. monocytogenes* from
air or equipment. In this situation, the extent of inactivation will depend on residual antimicrobial solution on the surface of the frankfurters.

The activity of 0.5% SLS was enhanced when it was combined with LA, and initial population of the pathogen decreased 1.9-2.0 log CFU/frankfurter after 24-h storage. The solution containing 5% LA and 1% of SLS had even greater antimicrobial effect on *L. monocytogenes*, reducing initial population of the pathogen by 3.7-3.9 log CFU/link, after 24 hours of storage (Fig.1). Increased effectiveness of SLS at a lower pH is in agreement with studies of Hill and Ivey (24), Restanio et al. (39), and Tamblyn and Conner (52).

Figure 2 shows numbers of *L. monocytogenes* on frankfurters (without sodium lactate) that were immersed for 1.0 min in treatment solutions that stored at 4°C for 90 days. After an initial lag phase for about 14 days numbers of *L. monocytogenes* in control frankfurters increased and reached almost 10 log CFU/link at day 90. A similar trend was observed in samples dipped in 0.5% SLS. The use of 1% SLS increased the lag phase of the pathogen to 28 days but subsequent rate of growth seemed to be similar to that of the control. This observed extension in lag phase of growth by 1% SLS may be attributed to sublethal injury of the survivors that has to be repaired before the pathogen can resume growth. The process of repair takes time and usually results in an extension of the lag phase of growth (6, 34). Treatment of frankfurters with 5% LA was very inhibitory to *L. monocytogenes*. The lag phase of growth of the pathogen was extended to 56 days. While the addition of SLS (0.5% or 1%) to 5% LA dramatically reduced initial numbers of *L. monocytogenes* on frankfurters these treatments failed to inhibit growth of survivors.
This strong observed growth inhibitory effect of lactic acid is not surprising. Similar to other organic acids, lactic acid is believed to inhibit microbial growth by diffusing the proton motive force (PMF) across the bacterial cytoplasmic membrane (18). This diffusion of PMF makes it difficult for the organisms to make energy and multiply because most of its adenosine triphosphate (ATP) is used up in pumping protons out of the cell. Lactic acid sprays or dips at 0.2 to 2.5% have been shown to inhibit growth of spoilage bacteria in various meat products and extend their shelf life (15, 49).

After initial lag for 14 days, *L. monocytogenes* in control samples increased and reached 9-10 log CFU/link at 90 days (Fig. 2). As presented on Fig. 3, the sodium lactate used in the formulation did not improve the initial decrease of *L. monocytogenes* after 24 hours of storage when combined with other antimicrobials (*P >* 0.05), however growth of the pathogen was completely suppressed during the entire storage period on frankfurters formulated with 2% SL. These results agree with those of previous research (29, 36, 42) that demonstrated inhibition of *L. monocytogenes* by sodium lactate in cured meat products. For example, Porto et al. (36) reported no growth of *L. monocytogenes* in beef/pork frankfurters prepared with potassium sorbate and stored for 90 days at 4°C.

Figure 5 shows numbers of *Listeria monocytogenes* on frankfurters (formulated with SL) that were dipped for 3.0 min in antimicrobial solutions, inoculated, then vacuum packaged and stored at 4°C for 90 days. During the entire storage period no growth of *L. monocytogenes* was observed on frankfurters irrespective of dipping treatment. Viable numbers of the pathogen on frankfurters
that were surface-treated with 5% LA, 0.5% SLS, and 1.0% SLS were reduced by 0.85, 1.2 and 1.3 log, respectively, at 90 days. As early as 28 days numbers of *L. monocytogenes* survivors on frankfurters immersed in 5% LA+ 0.5% SLS or 5%LA+ 1% SLS deceased by 0.8 to 1.1 log-cycles then numbers remained relatively constant through 90 days.

While previous studies have reported on the listeriostatic effect of SL added to RTE processed meat products (1, 3, 23, 43, 46), the reduction in viability of the pathogen observed in the present study may be attributed to the combined antibacterial action of surface treatments and that of the 2% SL in the frankfurter formulation. Earlier studies have reported reductions in the viability of *L. monocytogenes* in meat products formulated with SL alone or combined with sodium diacetate (SDA) and dipped in organic acids. For example, approximately 0.6 to 1.0 log CFU/cm² reduction of numbers of *L. monocytogenes* were observed on frankfurters formulated with SL (1.8%) and SDA (0.125 or 0.25 %) and dipped in lactic or acetic acid (2.5%) (2).

These results as well as those reported in the present study are consistent with the concept of multiple barriers or hurdle technology whereby antimicrobials applied in food preservation may be more effective in multiple than in single form by inflicting simultaneous and variable injuries in foodborne bacteria (27, 50). On this basis, bacterial maintenance of metabolic activities while repairing multiple injuries requires the activation of many different cellular mechanisms. The activation of cellular mechanisms to facilitate repair of a variety of injuries can be very
energetically demanding for sub-lethally injured cells and consequently result in loss of viability in bacterial cells (27).

Table 1 shows the pH values of frankfurters (with or without added SL) that were immersed for 3.0 min in antimicrobial solutions, inoculated and stored vacuum-packaged at 4°C for 90 days. The pH of all frankfurter samples, regardless of treatment, was within the pH growth range for \emph{L. monocytogenes}. There was no significant difference in initial pH (~6.14) of water-dipped (control) frankfurters whether or not SL was present in the product formulation \((P > 0.05)\). These results are consistent with those of Bloukas et al. (4) who researchers reported no differences in pH of frankfurters prepared with or without 2% of SL. Also, other previous studies confirmed that SL does not affect the pH of RTE meats (5, 10, 26, 54).

The addition of SL to the formulation of frankfurters did not significantly change the pH of the product during storage. In previous studies it was suggested that the relatively stable pH of frankfurters made with SL and stored at refrigeration temperatures was attributed to growth inhibition of lactic acid bacteria (14, 33). In contrast, control frankfurters that were not formulated with SL exhibited significant reduction in pH by day 90; the pH decreased from 6.14 to 5.75 \((P < 0.05)\). This is not surprising because frankfurters without SL supported rapid growth of \emph{L. monocytogenes} survivors with numbers reaching about 9.7 log CFU per frankfurter at day 90 (Figure 4). The growth of psychrotrophic lactic acid bacteria in the frankfurters may have resulted in acid production and concomitant decrease in pH (4, 43).
The initial pH of frankfurters dipped in 5% LA + SLS (0.5 or 1%) decreased by about 0.27 to 0.29 units. Frankfurters subjected to these treatments exhibited no significant differences in pH throughout 90 days of refrigerated storage. Compared to control frankfurters, those treated with 5% LA + SLS (0.5 or 1%) did not show significant decreases in pH during storage whether or not they were formulated with 2% SL. These results are likely due to the inhibition of lactic acid bacteria by SLS in frankfurters.

No significant differences in Hunter $L$, $a$ and $b$ color values were observed for vacuum packaged frankfurters (formulated with or without SL) during storage at 4°C for 90 days (data not shown). Bloukas et al. (4) also reported that incorporation of 2% of SL in frankfurters did not affect external and internal color of frankfurters during storage for 6 weeks at 4°C. However, Choi and Chin (10) reported an increase in yellowness and decrease in lightness of frankfurters prepared with or without SL during 6 weeks storage at 4°C.

While fresh meats usually become discolored after treatment with organic acids (35, 47) cured meats seem to resist marked changes in color from treatment with organic acids. For example, in the present study, the surface treatment with LA and SLS did not affect Hunter color $L$, $a$, and $b$ values of the frankfurters ($P>0.05$) during storage. Also, Sommers et al. (51) surface treated frankfurters with 5% (w/v) citric acid solution and demonstrated no effect on color. This observed surface color stability of frankfurters surface-treated with organic acids may be due to presence of nitrosohemochrome, the stable pink pigment of cured meat.
In summary, the results of the present study indicate that combinations of GRAS chemical antimicrobials applied on the surface of frankfurters formulated with 2% SL may provide much better control of *L. monocytogenes* than the sole use of antimicrobials on the product surface or in the formulation. Specifically, the results demonstrate that a combination of 5% LA + 1% SLS (bactericidal) as a surface treatment and 2% SL (bacteriostatic) in the meat formulation could provide substantial protection of frankfurters against *L. monocytogenes*. This control strategy has good potential for use in RTE meats to control *L. monocytogenes* and meet the current requirements of interim final rule (53). Further research is needed to optimize concentrations of GRAS antimicrobials for use in a hurdle technology approach to control *L. monocytogenes* in RTE meats without altering positive quality characteristics of these meat products.
Table 1. *The pH values during the storage of frankfurters made with or without sodium lactate and dipped for 3 minutes in lactic acid alone or in combination with sodium lauryl sulfate.*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Day 0</th>
<th>Day 28</th>
<th>Day 90</th>
<th>SEM</th>
</tr>
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<tbody>
<tr>
<td>H₂O (no SL)</td>
<td>$\lambda 6.14^x$</td>
<td>$\lambda 6.03^x$</td>
<td>$\lambda 5.75^y$</td>
<td>0.04</td>
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<tr>
<td>H₂O(2%SL)</td>
<td>$\lambda 6.13^x$</td>
<td>$\lambda 6.07^x$</td>
<td>$\lambda 6.01^x$</td>
<td>0.02</td>
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<tr>
<td>5%LA+ 5%LA+1%SLS (2%SL)</td>
<td>$\beta 5.85^x$</td>
<td>$\beta 5.85^x$</td>
<td>$\lambda 5.71^x$</td>
<td>0.05</td>
</tr>
<tr>
<td>1%SLS (no SL)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>0.05</td>
<td>0.02</td>
<td>0.07</td>
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*SEM is standard error of the means*

Mean values with different subscripts (A, B) within a column are significantly different ($P< 0.05$)

Mean values with different superscripts (x, y) within a row are significantly different ($P< 0.05$); n=3
Table 2. Hunter color values during the storage of frankfurters made with or without sodium lactate and dipped for 3 minutes in lactic acid alone or in combination with sodium lauryl sulfate.

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<tr>
<th>Hunter color value</th>
<th>Day</th>
<th>H₂O</th>
<th>H₂O+2% SL</th>
<th>5%LA+1%SLS</th>
<th>5%LA+1%SLS+2%SL</th>
<th>SEM</th>
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<tr>
<td>L-value 0</td>
<td>0</td>
<td>56.66</td>
<td>55.96</td>
<td>54.76</td>
<td>55.20</td>
<td>0.54</td>
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<td></td>
<td>42</td>
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<td>57.10</td>
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<td>90</td>
<td>56.51</td>
<td>57.64</td>
<td>56.92</td>
<td>56.94</td>
<td>0.30</td>
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SEM is standard error of the mean; n=3
Figure 1. *Listeria monocytogenes* survivors on frankfurters (w/o sodium lactate) at 24 hours after the product was immersed (1.0 or 3.0 min) in H$_2$O or antimicrobial solutions, inoculated, then vacuum packaged and held at 4°C. 

The error bars represent the standard error of mean; n=3
**Figure 2.** Numbers of *Listeria monocytogenes* on frankfurters (without sodium lactate) that were immersed for 1.0 minute in water (control) or solutions of lactic acid (LA), sodium lauryl sulfate (SLS) or combination of LA and SLS, vacuumpackaged and stored at 4°C. *Symbols:* ●, control; □, 5% LA; ○, 0.5% SLS; △, 1% SLS; •, 5% LA +0.5% SLS; ■, 5% LA +1% SLS.

* Least significant difference; n=3
Figure 3. Numbers of *Listeria monocytogenes* on frankfurters (with sodium lactate) that were immersed for 1.0 minute in water (control) or solutions of lactic acid (LA), sodium lauryl sulfate (SLS) or combination of LA and SLS, vacuumpackaged and stored at 4°C. Symbols: ᴬ, control; □, 5% LA; ○, 0.5% SLS; △, 1% SLS; ●, 5% LA + 0.5% SLS; ■, 5% LA + 1% SLS

* Least significant difference; n=3
Figure 4. Numbers of *Listeria monocytogenes* on frankfurters (without sodium lactate) that were immersed for 3.0 minutes in water (control) or solutions of lactic acid (LA), sodium lauryl sulfate (SLS) or combination of LA and SLS, vacuumpackaged and stored at 4°C. Symbols: •, control; □, 5% LA; O, 0.5% SLS; △, 1% SLS; ●, 5% LA +0.5% SLS; ■, 5% LA +1% SLS

* Least significant difference; n=3
Figure 5. Numbers of *Listeria monocytogenes* on frankfurters (with sodium lactate) that were immersed for 3.0 minutes in water (control) or solutions of lactic acid (LA), sodium lauryl sulfate (SLS) or combination of LA and SLS, vacuumpackaged and stored at 4°C. *Symbols:* ●, control; □, 5% LA; ○, 0.5% SLS; △, 1% SLS; ●, 5% LA + 0.5% SLS; ■, 5% LA + 1% SLS.

* Least significant difference; n=3
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postprocess contamination and extended storage of frankfurters at $4^\circ$C in vacuum packages. J. Food Prot. 65:299-307.


CHAPTER 4. IMPACT OF SODIUM LACTATE, LACTIC ACID, SODIUM LAURYL SULFATE AND ELECTRON BEAM IRRADIATION ON VIABILITY OF LISTERIA MONOCYTOGENES AND QUALITY OF VACUUM-PACKAGED FRANKFURTERS

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Abstract

The effect of sodium lactate (SL), lactic acid (LA), sodium lauryl sulfate (SLS) and electron beam irradiation on viability of *Listeria monocytogenes* and selected quality characteristics of vacuum-packaged frankfurters was evaluated. Frankfurters were dipped for 1 minute in distilled water (control) or in a solution of containing 5% (v/v) LA and 1% (w/v) SLS, then drained for one minute and placed into sterile vacuum packaging bags. The treated product was surface-inoculated with $10^8$ CFU of a five-strain mixture of *L. monocytogenes* per link, vacuum packaged, irradiated (0.0, 1.0, or 2.0 kGy), and stored at 4°C for 90 days. Numbers of the pathogen on frankfurters surface-treated with 5% LA + 1% SLS were reduced by ~ 4.2 log CFU/link. Irradiation (2.0 kGy) further reduced numbers of the pathogen to non-detectable levels based on colony counts on MOX; however, in such instances, *L.
Listeria monocytogenes were always detected by enrichment. No growth of the survivors occurred during the entire storage period. The measured Hunter color values of the frankfurters were not affected by the treatments and did not change over the storage time. The volatile profile of surface treated frankfurters was not different from the control, and no increase in lipid oxidation products and sulfur compounds was observed after irradiation and during the storage. Based on these results electron-beam irradiation (1.0 or 2.0 kGy) of frankfurters formulated with SL (2%) and surface-treated with LA (5%) + SLS (1%) has a good potential for controlling L. monocytogenes in these popular RTE meat products.

Introduction

Listeria monocytogenes is a ubiquitous pathogenic bacterium that causes life-threatening illness in immuno-compromized humans. In the United States, foodborne disease linked to this pathogen causes about 2,500 illnesses annually. Compared to all known food-borne pathogens L. monocytogenes-associated foodborne disease causes the highest hospitalization rate (92%) and the second highest fatality rate (20%) (32). Not only is L. monocytogenes one of the most resistant non-spore-forming pathogens to food processing methods, but it can survive and multiply at refrigeration temperatures. Therefore, the growth of this pathogen in ready-to-eat (RTE) refrigerated foods with extended shelf-life poses a serious health risk to consumers.
Frankfurters are RTE meats that can be consumed without reheating. Among RTE meat products, they are the ones most often to become contaminated, have a higher degree of contamination, and usually have a larger serving size than other RTE meats. In addition, these meat products can easily support the growth of *L. monocytogenes*; therefore, they pose the greatest risk to consumer health (17). Because of the high mortality rate of listeriosis (20-30%) and the ability of the pathogen to proliferate during refrigeration storage, in 1989, the Food and Drug Administration (FDA) and U. S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) established a “zero tolerance” rule for *L. monocytogenes* in RTE meats (39). Implementation of this policy together with Hazard Analysis Critical Control Point (HACCP) system led to a significant reduction in the incidence of listeriosis in the 1990’s (30).

Although the incidences of foodborne listeriosis seem to have reached a plateau, there have been at least three major outbreaks of listeriosis associated with frankfurters and deli type meats in the United States in recent years. In 1998, an outbreak resulting in 101 cases of listeriosis, 20 deaths and was traced to contaminated frankfurters from a Michigan meat processing plant (9). In 2002, two separate listeriosis outbreaks were linked to sliced RTE turkey products (10, 13). During the same period several other large recalls of RTE meats and poultry products contaminated with *L. monocytogenes* have occurred, resulting in large economic losses to the food processing industry (44).
The current objective of the United States Department of Heath and Human Services is to reduce the incidence of listeriosis by 50% by the year 2010 (3). To improve safety of RTE meat products, the Food Safety and Inspection Service (FSIS) of the USDA issued an interim final rule for the control of *L. monocytogenes* in RTE meat and poultry products. The rule requires RTE meat and poultry processors to use one of three alternatives: a) post-lethality treatment of the product and antimicrobial agent or process that suppresses or limits growth; b) post-lethality treatment of the product and antimicrobial agent or process that suppresses or limits growth including a sanitation program; or c) a sanitation program only (18). RTE meats typically can be made safe using generally recognized as safe (GRAS) chemicals or ionizing radiation.

Antilisterial activity of lactic (LA) acid and sodium lactate applied as a part of formulation or as a post-lethal intervention has been investigated in various RTE meat products (6, 29, 34-36). These GRAS chemicals can suppress *L. monocytogenes* on RTE meats without causing substantial changes in quality of RTE meats (12, 42).

Sodium lauryl sulfate (SLS) may be safely used in food as an emulsifier, wetting and whipping agent, and surfactant in various foods (11). It can be also used as a detergent and antimicrobial agent. The antimicrobial properties of SLS increase in the pH range of 1.5 to 4.0 and at elevated temperatures (16). In 1989, Hill and Ivey patented a method for control *Salmonella* spp. on poultry carcasses using SLS in combination with lactic acid (21). Tamblyn and Conner (43) reported that 125 ppm
of SLS reduces the population of *Salmonella* Typhimurium on inoculated chicken skin by 1.3 log units when combined with 0.5% lactic acid. The antimicrobial properties of SLS as a component of commercial sanitizers for fresh produce has been well studied and widely employed (38).

Ionizing radiation is an effective physical intervention to control pathogens in RTE meats; however, it can negatively affect the odor of the product by the production of volatile compounds that are generated as a result of the degradation of amino acids and unsaturated fatty acids (1). Sulfur volatiles produced by irradiation of meat products are usually retained in vacuum packed products and that odor is described as “barbequed-corn-like” (2, 25).

To our knowledge, there is no published research on the combined effect of SLS, LA and ionizing radiation on *L. monocytogenes* in frankfurters. Thus, the objective of this study was to evaluate the combined effectiveness of LA + SLS (as a surface treatment) and electron beam irradiation, for destroying *L. monocytogenes* on the surface of frankfurters formulated with 2% SL. An additional objective was to evaluate the impact of the combined treatments on color and off odor volatiles in the product.
Materials and methods

Experimental design

Two dipping solutions including the control, seven storage periods (1, 14, 28, 42, 56, 70, and 90 days) at 4°C and three irradiation doses (0, 1.0 and 2.0 kGy) were used in present study. A randomized complete-block full factorial experimental design was used where the experimental unit was two frankfurters. Three replicates of the treatment were carried out.

Preparation of frankfurters

Frankfurters were prepared with pork fat trimmings (40/60 lean/fat ratio) and lean beef trimmings (80/20 lean/fat ratio) at Iowa State University Meat Laboratory. The meat was ground through 0.79-cm grinder plate, and divided into two batches. Sodium lactate, as a powder (SL; Purac Inc., Lincolnshire, IL) was added alone with salt, sodium erythrobate, sodium nitrite, seasoning and water with ice during emulsification in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA.). The meat batter was extruded through a meat stuffer (Risco® Model RS 4003-165; Stoughton, Mass., U.S.A.) into 22-mm peelable cellulose casings (Devro Teepack™ Wiene-Pack® Coastal corrugated Inc., N. Charleston, S.C., U.S.A.). The encased meat batter was linked at 14.0 cm length by 2.2 cm in diameter using a poly-clip system (Gmbh and Co.,KG, Frankfurt, Germany).

The linked product was hung on racks and cooked for 90 minutes using the conventional cooking-smoking cycle in a humidity-controlled smoke house (Alkar,
DEC Intl. Inc., Lodi, Wis., U.S.A.) to an internal temperature of 71.1°C. Natural smoke (hardwood sawdust; Frantz Co., Milwaukee, WI) was applied during the cooking cycle. At the end of the cycle frankfurters were showered with cool water, and then kept in a walk-in refrigerator at 4.0°C for 18-19 hours. The following day, frankfurters were peeled (Peeler Townsend 2600, Des Moines, IA) sealed in vacuum bags, then stored at -20°C in a walk-in refrigerator until used in the experiment.

**Treatment of frankfurters**

Frozen frankfurters for each replication were transferred to a walk-in refrigerator and thawed overnight at 4°C. After thawing, frankfurters were aseptically removed from the bulk package and placed into a sanitized aluminum basket (28 samples per basket) and immersed in freshly prepared solution (3 liters) 5% (vol/vol) lactic acid (LA; Birko Co. Denver, CO.) and 1% (wt/vol) sodium lauryl sulfate (SLS; Fisher Scientific, Fair Law, NJ), or distilled water (control) for 1 min at room temperature (23±1°C). After immersion, each basket of frankfurters was removed and placed over a plastic container to allow samples to drain for about 1 minute. Each frankfurter was placed in a separate vacuum bag (Cryovac B-2540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission=0.5-0.6g at 100°F, 100% RH/100 sq.in./24h; oxygen transmission rate=36 CC at 40°F/m²/24h/0%RH).
Preparation of the inocula

A five-strain mixture of *Listeria monocytogenes* was used to inoculate frankfurters in this study. The strains used were Scott A NADC 2045, 101 M, H7776, 108 M, and F 6854. The Scott A strain was obtained from the National Animal Disease Center (NADC) in Ames, IA whereas the other four strains were obtained from Dr. Peter Slade at the Illinois Institute of Technology in Chicago, IL. Stock cultures of *L. monocytogenes* were held at -70°C in brain heart infusion (Difco, Becton Dickson, Sparks, MD) with 10% glycerol until used for preparing working cultures. Working cultures of each *L. monocytogenes* strain were prepared by transferring a loopful of stock culture into 10 ml of tryptic soy broth (Difco) supplemented with 0.6% yeast extract (Difco; TSBYE) followed by incubation at 35°C for 18 hours. Prior to each replication of the experiment, two consecutive 18-h transfer of each *L. monocytogenes* strain were prepared in TSBYE at 35°C. A five-strain cocktail was prepared by transferring 6 ml of each culture into a sterilized 50 ml centrifuge tube. Cells were harvested by centrifugation (10,000xg, 10 min, 4°C) and the supernatant was discarded. Pelleted cells were suspended in 30 ml of 0.1% peptone water and washed by centrifugation. This procedure was repeated twice to obtain washed cells. The washed cell suspension was diluted 10-fold in order to obtain 10^8 CFU per ml, which served as the inoculum. Cell population in the inoculum was determined by plating on tryptic soy agar (TSA; Difco) supplemented with 0.6% yeast extract (TSAYE) plates and incubation at 35°C for 24 h.
**Inoculation of frankfurters**

Each frankfurter was spot-inoculated with 0.1 ml of the five-strain cocktail of *L. monocytogenes* to give initial population of $10^8$ CFU/link. Inoculated frankfurters were then massaged manually from outside of the bag for 10 sec. to spread the inoculum over that surfaces. Bags of inoculated frankfurters were vacuum packaged at 95 kPa with MultivacA 300/51 vacuum packaging machine (Multivac Sepp Haggenmuller, GmbLt & Co., Wolfertschwenden, Germany) and stored at 4°C for 90 days in a walk-in refrigerator.

**Irradiation of the frankfurters**

Vacuum sealed frankfurters were kept at 4°C until the next day when they were exposed to radiation at the Iowa State University Linear Accelerator Facility, which is equipped with a MeV CIRCLE III Linear Electron Accelerator (MeV Industrie S. A., Jouy-en-Josas, France). Three target average dose levels (0, 1.0, and 2.0 kGy) at dose rate 54.6 kGy/ min were applied to the samples at an energy level of 10 MeV. Target average doses are arithmetic averages of doses determined at the surfaces of the irradiated frankfurters.

Absorbed radiation doses were determined using alanine dosimeters which were read with 104 Electron Paramagnetic Resonance Instrument (Bruker Instruments Inc., Billerica, Mass.).
**Microbiological analysis**

Vacuum packaged frankfurters were opened aseptically and 20 ml of 0.1% sterile peptone water (Difco) were added to each package. Samples were vigorously massaged by hand from outside of the package for 30 seconds. Ten-fold serial dilutions of the wash solution were prepared in 0.1% of peptone water. Aliquots (0.1 ml) of appropriate dilutions were surface-plated onto modified Oxford agar (MOX; Difco). All inoculated agar plates were incubated at 35°C for 48 hours and typical colonies of *L. monocytogenes* were counted after incubation and expressed as log CFU per frankfurter.

**Color measurements**

The color of the frankfurters was measured at days 1, 42 and 90 on the surface of vacuum packaged uninoculated samples using a Hunter Labscan spectrophotometer (Hunter Associated Labs Inc., Reston, VA, USA) that had been calibrated against white and black reference tiles packaged in the same bags as those used for meat packaging. Hunter *L*- (lightness), *a*- (redness), and *b*- (yellowness) values were obtained using an illuminant A (light source). Area view and port size were 0.25 and 0.40 inch, respectively. The average value from two random locations on each sample surface was used for statistical analysis.
Determination of volatile compounds

A purge-and-trap apparatus (Solatek 72 and Concentrator 3100; Tekmar-Dohrmann, Cincinnati, OH, USA) connected to a gas chromatograph/mass spectrometer (HP 6890/HP 5973; Hewlett-Packard Co., Wilmington, DE, USA) was used to analyze volatiles produced. The minced frankfurter sample (2 g) was placed in a 40-mL sample vial, and the vial was flushed with helium gas (40 psi) for 5 s. The maximum waiting time of a sample in a refrigerated (4 °C) holding tray was less than 4 h to minimize oxidative changes before analysis. The meat sample was purged with helium gas (40 mL/min) for 15 min at 40 °C. Volatiles were trapped using a Tenax-charcoal-silica column (Tekmar-Dohrmann) and desorbed for 2 min at 225 °C, focused in a cryofocusing module (-80 °C), and then thermally desorbed into a capillary column for 60 s at 225 °C.

An HP-624 column (8.5 m x 0.25 mm i.d., 1.4 µm nominal), an HP-1 column (60 m x 0.25 mm i.d., 0.25 µm nominal; Hewlett-Packard), and an HP-Wax column (6.5 m x 0.25 mm i.d., 0.25 µm nominal) were connected using zero dead-volume column connectors (J &W Scientific, Folsom, CA, USA). Ramped oven temperature was used to improve volatile separation. The initial oven temperature of 0 °C was held for 1.5 min. After that, the oven temperature was increased to 15 °C at 2.5 °C/min, increased to 45 °C at 5 °C/min, increased to 110 °C at 10 °C/min, increased to 210 °C at 20 °C/min, and then held for 3 min at the temperature. Constant column pressure at 22.5 psi was maintained. The ionization potential of the mass selective detector (Model 5973; Hewlett-Packard) was 70 eV, and the scan range was 19.1 to 400 m/z. Identification of volatiles was achieved by comparing mass spectral data of
samples with those of the Wiley Library (Hewlett-Packard). Standards were used to confirm the identification by the mass-selective detector. The area of each peak was integrated using the ChemStation (Hewlett-Packard), and the total peak area (\( \text{pA} \times 10^4 \)) was reported as an indicator of volatiles generated from the sample.

**Statistical analysis**

Analysis of variance was used to assess the significance of the differences in the mean values found in the experiment. Three complete replicates of the experiment were conducted each with two samples per treatment. Differences among variables were tested for significance using Tukey's honestly multiple comparisons test and considered statistically significant at when the associated \( P \)-value was less than 0.05 (40).

**Results and discussion**

Figure 1 shows log reduction in populations of \( L. \) monocytogenes on frankfurters formulated with 2\% (w/w) sodium lactate, immersed for 1.0 minute in distilled water or 5\% LA + 1\% SLS, then irradiated with 1.0, or 2.0 kGy. Initial numbers of the pathogen recovered from frankfurters dipped for 1.0 minutes in distilled water ranged from 7.85 to 8.00 log CFU/frankfurter (data not shown). Initial numbers were reduced the by 1.8 and 4.0 log CFU/link, following irradiation with 1.0 and 2.0 kGy, respectively. Slightly different results were reported by other recent
published studies involving the irradiation of frankfurters. For example, Foong et al. (19) applied electron beam irradiation (2.0 to 4.0 kGy) to six different types of vacuum-packaged RTE meats including frankfurters inoculated with a five-strain mixture of *L. monocytogenes*. Those researchers reported reductions of 1.69 and 3.27 log CFU/link following irradiation of frankfurters with 1.0 and 2.0 kGy, respectively. Sommers et al. (42) treated frankfurters with gamma irradiation (2.0 kGy) and reported \(-3.3\) log reduction for a four-strain mixture of *L. monocytogenes*. These variations in the extent of reduction in numbers of *L. monocytogenes* in frankfurters after irradiation is not surprising considering the fact that many factors including microbial strains, food composition, pH, temperature during irradiation, and medium used to recover survivors, are known to affect microbial inactivation by irradiation (33).

The pH of the antimicrobial treatment solution used in the present study was 2.2. Surface treatment of the frankfurters with 5% LA + 1% of SLS alone reduced *L. monocytogenes* initially by 4.2 log CFU/link (Figure 1). Our observations are consistent with the concept of acid anionic surfactant technology in which anionic surfactants exhibit increased antimicrobial activity in an acidic environment (16).

Sodium lauryl sulfate (SLS) is an anionic surfactant that is characterized by a balance between a hydrophobic residue and a negatively charged hydrophilic group. The mechanism of action of anionic surfactants involves denaturation of cell proteins, enzyme inactivation, disruption of cell membranes and permeability (16). Tamblyn and Conner (43) used a solution of 125 ppm SLS and 0.5% of LA against *Salmonella* Typhimurium on surface of broiler chicken skins. When applied alone,
SLS (125 ppm) was not bactericidal; however, when SLS was combined with LA (0.5%) a 1.54 log reduction in firmly attached cells of the pathogen was observed following a 2.0 minute exposure of inoculated chicken skin to that treatment solution at 50 °C.

In the present study, although the temperature of the 5% LA +1%SLS treatment solution (23 ± 1 °C) and the exposure time for the frankfurters were lower than those used by Tamblyn and Conner (43), higher log reductions in bacterial numbers were achieved. Differences in these results may be attributed to both the type of food product tested as well as the bacterial type used in the studies. Chicken skin has a very high fat content and numerous crevices, folds, and feather follicles that can serve as physical barriers that protect bacterial cells from antimicrobial agents. In contrast, although the surface of a frankfurter is hydrophobic due to fat content, it is far smoother that that of chicken skin and may offer less physical protection to attached bacteria. Also, the greater sensitivity of *L. monocytogenes* observed in the present study is consistent with the fact that anionic surfactants such as SLS are more effective against gram-positive bacteria than gram-negative bacteria (5, 14).

Dipping of frankfurters in dipping in 5%LA +1%SLS followed by irradiation (1.0 kGy) reduced numbers of *L. monocytogenes* by 5.6 log CFU/link. When frankfurters were irradiated with 2.0 kGy after dipping, the pathogen was not detected (< 20 cells per frankfurter) by surface plating on MOX agar. The detection limit was 20 cells per frankfurter. In instances when *L. monocytogenes* was not detected by direct plating on MOX agar, the pathogen was almost always recovered
by enrichment in UVM broth (26). The sequential application of antimicrobial surface treatment and irradiation (1.0 or 2.0 kGy) yielded significantly greater reductions in numbers of the pathogen compared to the single use of either intervention ($P < 0.05$). These results clearly indicate the superior antimicrobial effectiveness of multiple interventions used for controlling pathogens in foods. Our results are also consistent with the concept of hurdle technology in which antimicrobial interventions may be more effective in multiple rather than in single form by creating simultaneous and variable injuries in bacterial cells (28, 41). In this regard, bacterial maintenance of homeostasis while repairing multiple injuries requires the activation of several different cellular mechanisms. The activation of cellular mechanisms for repair of a variety of injuries can be energetically demanding for sub-lethally injured cells and result in loss of viability in bacterial cells (28).

Figure 2 shows numbers of *L. monocytogenes* on frankfurters that were immersed (1.0 minute) in water or in 5% LA +1% SLS, vacuum-packaged, irradiated (0.0, 1.0, or 2.0 kGy) and stored at 4 °C for 90 days. After surface treatment and irradiation, no growth of *L. monocytogenes* occurred in frankfurters during the entire storage period. The observed growth inhibition of *L. monocytogenes* survivors is most likely linked to the presence of 2% SL in the frankfurter formulation. This result agrees with those of previous studies (7, 31, 35, 37), which showed that the incorporation of SL in RTE meat formulations inhibited growth of *L. monocytogenes* during storage at 4°C. In some instances limited growth inhibition or reduced viability of *L. monocytogenes* in RTE meats formulated with SL has been reported. For example, Glass et al. (20) observed that 2%SL restricted growth of *L.*
monocytogenes for only 28 days on smoked wieners stored at 4.5 °C. Bacus and Bonental (4) reported a listeriocidal effect of 2% SL in frankfurters during 60 days of storage at 4.4°C. The differences in antimicrobial effectiveness of SL against L. monocytogenes as reported in those studies may be attributed to the use of different meat product formulations (type of meat, lean/fat ration, amount and type of salts and spices).

Table 1 shows the volatile profile of vacuum packaged frankfurters used in the present study. The total number volatiles (39) identified by GC-MS were placed into five groups for ease of reference. The volatile profile of irradiated frankfurters dipped in 5% LA + 1% SLS was not significantly different from those dipped in distilled water (P>0.05). Irradiation of frankfurters with either dose did not significantly affect the amount of sulfur-containing volatiles (S-volatiles) produced. Previous studies reported increased production of S-volatiles, major volatile compounds that contributed to irradiation odor, as a result of the degradation of sulfur containing amino acids in meat products (1, 2, 25). Increased amount of S-volatiles after irradiation (3.0 kGy) of chicken breast fillets was reported by Du et al. (15). Zhu et al. (47) irradiated turkey ham with 1.0 and 2.0 kGy and reported an increased amount of dimethyl disulfide. They also concluded that sulfur-related off-odor was significantly higher in irradiated samples, than non-irradiated samples, as determined by sensory evaluation.

The differences in the levels of production of S-volatiles in the present study compared to those of previous studies may be due differences in type of meat products used in experiments. Although it is well documented that ionizing radiation
can produce these S-volatiles in meats, it is likely that the extent of production may be reduced in cured meat products such as frankfurters. For example, Houser et al. (2004) irradiated several RTE meat products with 1.6 kGy and detected elevated S-volatile levels for all samples except beef frankfurters, where sulfur compounds, including dimethyl disulfide, decreased as a result of irradiation. The reason for decrease of S-volatiles in frankfurters after irradiation is not clear and warrants further investigation.

Irradiation processing did not seem to affect aldehydes and ketones, the lipid oxidation-dependent volatiles in our experiment. However, Zhu et al. (47) reported that the content of hexanal and pentanal and 2,3-butanedione was increased in turkey ham by irradiation with 1.0 or 2.0 kGy. Houser et al. (23) reported that all of the aldehydes, ketones and alcohols increased in various vacuum packed RTE meats, including frankfurters, as a result of a 1.6 kGy dose. Our results partially agree with a recent study by Lee and Ahn (27) who reported that irradiation of turkey breast rolls with 3.0 kGy increased production of ketones, whereas the amount of aldehydes was not changed by irradiation. Alcohols in our samples were not affected by irradiation which also agrees with study of Lee and Ahn (27). In present study, levels of hydrocarbons increased by irradiation, whereas this increase was dose-dependant and consistent at all storage periods.

The amount of terpenoids, volatile compounds produced by frankfurter spice blends did not seem to be affected by irradiation ($P>0.05$). In contrast, Houser et al. (23) reported increased amounts of terpenoids in pork frankfurters irradiated at 1.6 kGy. This difference may be due differing composition and amount of spice added to
the frankfurters. Amounts of other volatile compounds did not change as a result of irradiation but they did decrease during storage ($P<0.05$).

In general, volatile profile did not change during storage with some exceptions. For example, hydrocarbons of non-irradiated control samples decreased after 42 days of storage. The results of our study indicate that irradiation of frankfurters at 1.0 or 2.0 kGy did not produce significant changes in product composition (data is not shown) and amount of volatile compounds.

The surface hunter color values of vacuum packaged frankfurters were compared based on storage time, treatment with 5% LA+1% SLS, and irradiation dose (Table 2). Dipping frankfurters in 5% LA+1% SLS or irradiating at 1.0 or 2.0 kGy did not affect measured Hunter color values of the frankfurters ($P>0.05$). This observation is in agreement with the findings of Houser et al. (23), who irradiated (1.6 kGy) various RTE meats including all-turkey, all-beef and all-chicken frankfurters, where they and observed no color difference between irradiated and non-irradiated products. In a separate study, Houser et al. (24) reported that irradiating vacuum packaged frankfurters at the same dose did not affect the internal color of the product. In an earlier study, frankfurters that were dipped in 0, 1, 5, or 10% citric acid then treated with gamma radiation developed statistically significant color changes ($P<0.05$); however, these changes were visually imperceptible to authors (42). In the present study, color of frankfurters did not change during the storage, which is consistent with results of Bloukas et al. (8). Those authors reported no color changes of frankfurters (formulated with 3.0 % SL) during 5 weeks of storage (4°C). However, Houser et al. (22) reported a reduction for $b$ values of
raw cured or uncured cooked ham, with no effect on cured cooked ham over 90 days storage period.

Conclusions

The contamination the frankfurters before packaging in the processing plant is of major concern. The combination of 1% SLS and 5% LA had a substantial synergistic, inhibitory effect against *L. monocytogenes*. The incorporation of sodium lactate in frankfurters, and surface treatment with sodium lauryl sulfate and lactic acid, followed by electron-beam irradiation, represent an effective strategy for controlling *L. monocytogenes* in this RTE product without negatively affecting product quality. This food preservation system could potentially be used by RTE meat processors to meet the USDA interim final rule alternative 1 and 2 criteria for controlling *L. monocytogenes* on RTE meats (45).
Table 1. Volatile compounds of vacuum packed frankfurters

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<th>Day</th>
<th>H2O</th>
<th>H2O+1kGy</th>
<th>H2O+2kGy</th>
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<td>120</td>
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^x,y=Values in the same column with the same superscript letter are not significantly different (P>0.05)
^A,B,C=Values in the same row with the same superscript letter are not significantly different (P>0.05)
SEM is standard error of the means; n=3
### Table 2. Hunter color values of vacuum packaged frankfurters, surface treated, irradiated and stored at 4 °C

<table>
<thead>
<tr>
<th>Hunter color value</th>
<th>Day</th>
<th>H₂O+ 0 kGy</th>
<th>H₂O+ 1kGy</th>
<th>H₂O+ 2 kGy</th>
<th>LA+SLS +0kGy</th>
<th>LA+SLS+ 1kGy</th>
<th>LA+SLS+ 2 kGy</th>
<th>SEM</th>
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</table>

*SEM* is standard error of the means; n=3

*xy* = Values in the same column with the same superscript letter are not significantly different (*P* > 0.05)
Figure 1. Log reduction in numbers of *Listeria monocytogenes* on frankfurters immersed for 1.0 minute in H$_2$O (control) or 5% LA+ 1% SLS followed by irradiation with 0, 1.0 or 2.0 kGy.

Individual columns represent log CFU/frankfurter reduction of 5 strains of *L. monocytogenes*. Each treatment expressed negligible changes during 90 days storage. Error bars represent standard error of the means.

Irradiation of the frankfurters treated with 5% LA+ 1% SLS followed by irradiation with 2.0 kGy reduced numbers of the pathogen below detectable levels based on colony counts; however, *L. monocytogenes* was consistently detected by enrichment of the samples during the storage time.
Figure 2. Numbers of *Listeria monocytogenes* on frankfurters that were immersed for 1.0 minute in water (control) or in 5% LA and 1% SLS, vacuumpackaged irradiated (0.0 or 1.0, kGy), and stored at 4°C. *Symbols:* ●, control; □, 1 kGy; ○, 2 kGy; △, LA+SLS; •, LA+SLS+ 1 kGy

Control (●), 1 kGy (□), 2 kGy (○), LA+SLS (△), LA+SLS+ 1 kGy (●),

* Least significant difference


by sodium diacetate and sodium lactate on wieners and cooked bratwurst. *J. Food Prot.* 65:116-123.


CHAPTER 5. GENERAL CONCLUSION

The present study confirms that frankfurters can readily support the growth of \textit{L. monocytogenes}, allowing populations of the pathogen to reach more than 9 log CFU/link. However, addition of 2% sodium lactate (SL) to frankfurter formulations prevents growth of the pathogen during 90 days of storage at 4°C.

When used alone as a surface treatment, lactic acid (LA) has a negligible bactericidal effect on \textit{L. monocytogenes} on frankfurters. This demonstrates inefficiency of LA as a surface treatment of frankfurters under the conditions of this study.

The generally regarded as safe surfactant, emulsifier and a mild antimicrobial, sodium lauryl sulfate (SLS), can be potentially used as a strong inhibitor of \textit{L. monocytogenes} on frankfurters if used in combination with lactic acid (5%) which lowers the pH.

Irradiation is effective in reducing numbers of \textit{L. monocytogenes} on frankfurters; however, survivors will eventually grow to high numbers if no additional antimicrobial agent is present to prevent growth.

The prior surface-treatment of frankfurters with antimicrobial chemical solutions to substantially reduce numbers of \textit{L. monocytogenes} facilitates the use of relatively low doses of irradiation for destruction of the pathogen on this RTE meat product.

A multiple hurdle approach involving surface-treatment of frankfurters with a chemical antimicrobial combined with irradiation and an inhibitory agent in the
product formulation has good potential for effectively controlling \textit{L. monocytogenes} in this RTE product without compromising product quality.

The results of the study can help RTE meat processors to meet current requirements of regulatory agencies, make their product safe to consumers and avoid costly recalls. However, concentrations and conditions of use of the described antimicrobials need to be validated for each type of RTE meat product.
ACKNOWLEDGMENTS

It was a great experience to study in Iowa State University. It’s a great privilege to be here, and I am thankful to all great people who helped me to succeed.

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And last but not the least I’d like to thank my wife Aliona for her love and patience.