Destruction of Listeria monocytogenes by electron-beam irradiation and food-grade chemicals in vacuum-packaged frankfurters stored at 4° or 10°C

Lawrence Cobb
Iowa State University

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Destruction of *Listeria monocytogenes* by electron-beam irradiation and food-grade chemicals in vacuum-packaged frankfurters stored at 4° or 10 °C

by

Lawrence Cobb

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

MASTER OF SCIENCE

Major: Microbiology

Program of Study Committee
Aubrey Mendonca (Major Professor)
   James Dickson
   Joseph Sebranek

Iowa State University
Ames, Iowa

2005

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Graduate College
Iowa State University

This is to certify that the Master’s thesis of
Lawrence Cobb
has met the thesis requirement of Iowa State University

Signatures have been redacted for privacy
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CHAPTER 1. GENERAL INTRODUCTION

INTRODUCTION

Listeria monocytogenes is a foodborne pathogen of major concern to food processors, federal regulatory agencies and consumers because of its high fatality rate (20%) in humans and its ability to grow at refrigerator temperatures. L. monocytogenes is ubiquitous in the natural environment and commonly found in food processing plants. Its ubiquity in the environment makes it challenging to prevent its transmission from raw animal products to meat processing environments and from the processing environment to ready-to-eat (RTE) meats. Food-related listeriosis outbreaks are usually associated with RTE foods such as luncheon meats, hot dogs, smoked fish, and some types of soft cheeses. There is an urgent need for post-processing interventions to destroy this pathogen in RTE meats.

The microbial safety of RTE meats may be enhanced by incorporation of foodgrade antimicrobials such as lactates, sorbate, and diacetate in meat formulations or applying them to RTE meat surfaces to retard or prevent growth of L. monocytogenes. Other antimicrobial food additives might have good potential for controlling L. monocytogenes in RTE meat products. For example, liquid smoke which contains aldehydes, acetic acid, creosote, and phenolic compounds, is strongly inhibitory to L. monocytogenes. Sodium lauryl sulfate (SLS) is an anionic surfactant that has a variety of food applications including uses as whipping agent, an emulsifier, and a partitioning agent. The antibacterial action of SLS presumably involves denaturing cell proteins, inactivating enzymes, and altering cell membrane permeability.

Another approach for controlling L. monocytogenes in RTE meats is the use ionizing radiation. Although relatively high doses of irradiation can eliminate foodborne pathogenic
and spoilage microorganisms, they are likely to produce undesirable sensory changes in food products. Therefore, application of antimicrobial food additives and irradiation to provide a hurdle technology approach will allow the use of lower irradiation doses while providing an effective way to control foodborne pathogens.

The major objective of the studies described in this thesis was to evaluate the combined efficacy of antimicrobial food additives and electron-beam irradiation to control \textit{L. monocytogenes} in frankfurters.

\textbf{THEESIS ORGANIZATION}

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

\textbf{LITERATURE REVIEW}

\textit{Listeria monocytogenes}

\textbf{Brief History}

\textit{Listeria monocytogenes} was first described in 1926 by Murray et al (1926) as the cause of death of laboratory rabbits and guinea pigs. The bacterium was isolated from the animals and the scientists discovered that a unique characteristic of the infection was the production of large mononuclear leucocytosis. Because the organism had not been previously
described, they named the bacterium *Bacterium monocytogenes*. At around the same time, Pirie (1927) isolated and described the same organism from gerbils in South Africa. He named the organism *Listerella hepatolytica* and later recommended the name be changed to *Listeria monocytogenes* in 1940.

**Morphology and Growth Requirements**

*Listeria monocytogenes* is a gram-positive, facultatively anaerobic, non-sporeforming rod (Salyers and Whitt, 2002; FAO/WHO, 2004; Rocourt, 1999). The rods are small (0.5 µm in diameter and 1-2 µm in length) with rounded ends and *L. monocytogenes* expresses a typical tumbling motility at 20-25°C, but not at 35°C (Rocourt, 1999). As for growth requirements, the three main parameters that influence growth of the organism are temperature, pH, and water activity (a<sub>w</sub>). *L. monocytogenes* is psychrotrophic and can grow at a temperature range of 0-45°C with an optimum temperature of 37°C (FAO/WHO, 2004). This was explained better when Junttila et al. (1988) discovered the minimum growth temperature of 78 strains of the bacterium was 1.1°C ±0.3°C. These researchers also observed that two strains grew at 0.5°C and eight strains grew at 0.8°C. *L. monocytogenes*’ optimum pH range for growth is 6-8. The range of growth, however, can be as low as 4.1 to as high as 9.6 depending upon the growth temperature which can range from 1°C-45°C (Jay, 2000). Finally, the pathogen grows optimally at a<sub>w</sub> of approximately 0.97. However, the pathogen can grow when the a<sub>w</sub> is as low as 0.90.

**Distribution**

*L. monocytogenes* is ubiquitous in the natural environment and can be found in decaying vegetation, soils, animal feces, sewage, and water. Typically, where you would expect to find lactic acid bacteria, *Brochothrix*, cornyeform bacteria, you most likely would
find *Listeria* (Jay, 2000). Weis and Seeliger (1975) isolated the organism from 8.4% to 44% of the samples taken from grain fields, pastures, mud, animal feces and wildlife feeding grounds. In Denmark, 15% of 75 silage samples were positive for *L. monocytogenes* as were 52% of 75 fecal samples from cows (Skovgaard and Morgen, 1988). Also, it is well known that foods of animal origin might carry any varying amounts of the pathogen. The USDA-FSIS monitoring/verification program monitored the incidence of *L. monocytogenes* for cooked and ready-to-eat meats between the years of 1993-1996 with incidences ranging from 0 to 8.1% (Farber and Peterkin, 1999).

**Listeriosis**

The human disease caused by *L. monocytogenes* is referred to as listeriosis. Epidemiological tests have shown that listeriosis is a foodborne disease (Slutsker and Schuchat, 1999). Unlike many other foodborne diseases, listeriosis is associated with a mortality rate of 20-30% (FAO/WHO, 2004; Slutsker and Schuchat, 1999).

The deaths that result from listeriosis involve fetuses, newborns, and/or immunocompromised adults. In pregnant women, *L. monocytogenes* crosses the placenta and can infect the fetus. An infected pregnant woman could experience mild flu-like symptoms, but if her fetus is infected a miscarriage, stillbirth, or premature birth would occur (FAO/WHO, 2004; Salyers and Whitt, 2002). In immunocompromised adults, the first indication of an infection is a fever, which is often followed by other disorders such as bacteraemia, meningitis, encephalitis, and malignancies (Rocourt and Cossart, 1997; Slutsker and Schuchat, 1999). *L. monocytogenes* will cause invasive listeriosis by penetrating the lining of the gastrointestinal tract. As the pathogen penetrates the intestinal tissue, phagocytes take up the bacteria which eventually escape from membrane-bound vacuoles and proceed to
grow in the cytosol. It is believed that phagocytes are the means of transportation for the pathogen to other parts of the body (Shelef, 1989; Farber and Peterkin, 1991).

**Outbreaks**

The first case of listeriosis was not reported until 1953 after a woman gave birth to stillborn twin. This was attributed the mother drinking raw milk from a cow with listeral mastitis (Potel, 1953). The full recognition of food being the primary source human exposure to *L. monocytogenes* did not occur until several large outbreaks of listeriosis occurred in North America and Europe during the 1980s (Broome et al., 1990; Bille, 1990). In recent years there have been other outbreaks of listeriosis involving ready-to-eat meats. In 1998, there was a multistate outbreak of listeriosis in which 40 illnesses were reported. Implicated *L. monocytogenes* isolates were of serotype 4b and shared an unusual pattern when they were subtyped by pulsed-field gel electrophoresis or by ribotyping methods. As a result four deaths occurred including three elderly people and one fetus. The outbreak was traced back to the consumption of cooked hot dogs manufactured by Bil Mar Foods (CDC, 1998). In 2002, there were 46 culture-confirmed cases resulting from *L. monocytogenes* infections. Seven deaths, and three stillbirths or miscarriages were linked to the consumption of sliceable turkey deli meat in several northeastern states. The definite source of the outbreak was not determined because isolates from the food product were different from those isolated from the patients. The manufacturer, Pilgrim’s Pride Foods recalled 27.4 million pounds of their products.

Also, there was a report in August of 2005 of a possible outbreak due to listeriosis in Houston, TX. The main source is illegally imported meat and dairy products from Mexico. Of the six cases reported, all were of Hispanic origin. Those infected were three pregnant
women, two newborns, and one elderly woman. The patients were all hospitalized and all made a recovery. Three cases were also reported earlier in the summer of 2005; so far no deaths occurred (Hopper, 2005).

**Prevention and Treatment**

Individuals who are most at risk (immunocompromised and pregnant women), should avoid consumption of soft cheeses, thoroughly heat ready-to-eat foods such as frankfurters and leftover foods until steaming hot, and reheat cold cuts before eating. Food processors have developed HACCP (Hazard Analysis Critical Control Points) plans to reduce the likelihood of future outbreaks (Salyers and Whitt, 2002; Slutsker and Schuchat, 1999). Once an individual has been promptly diagnosed in time with listeriosis, antibiotic treatment can alleviate the worst effects of the infection. Ampicillin or penicillin is most recommended.

**Organic Acid Salts**

**Sodium lactate**

\[
\text{Na}^+ \quad \text{CH}_3\text{CH(OH)CO}_2\text{Na}
\]

**General characteristics:**

Sodium lactate was approved by the USDA as a flavor enhancer with use up to 2% (Federal Register, 1990). It has been shown by increasing the levels of sodium lactate greater than 2% increases the lag phase and delays the logarithmic phase of microbial growth (Miller, 2005). Miller also states that the addition of sodium lactate improves the overall meat color, increases the meat juiciness, and may indirectly aid in limiting lipid oxidation.
Mode of Action:

Miller (2005) reported the mode of action of sodium lactate. The form of sodium lactate that has good antimicrobial properties is lactic acid which can be incorporated into the cell. Lactic acid then disrupts the cell’s metabolism and by diffusing the proton motive force which consequently inhibits ATP synthesis. Adding sodium lactate to meat products lowers the water activity which also slows microbial growth.

Characteristics in meat products:

Shelef and Yang (1991) observed the effects of sodium and potassium lactate in tryptic soy broth, chicken, and beef. Concentrations more than 5% delayed growth of *Listeria monocytogenes* in broth. However, a 4% concentration of the lactates suppressed growth in chicken and beef. Growth suppression was increased as storage temperature decreased. Another study assessed the effects of sodium lactate to control *L. monocytogenes* in raw salmon. Other food additives used were sodium chloride and sodium nitrite (Pelroy et al., 1994). Antilisterial effects of sodium lactate were increased when this organic salt was used in combination with nitrite, NaCl, or both. At 5°C, 2% sodium lactate and 3% NaCl inhibited *L. monocytogenes* growth for 50 days. When stored at 10°C, 3% sodium lactate with 3% NaCl or 2% sodium lactate with 3% NaCl and sodium nitrite (125 ppm) inhibited growth for 35 days.

Nykanen et al. (2000) observed the behavior of *L. monocytogenes* in cold smoked rainbow trout and its inhibiton by sodium lactate and nisin. Nisin (4000-6000 IU/ml) and sodium lactate (60%) were injected individually or in combination before or after the smoking process. The samples were inoculated with $10^3$-$10^4$ log CFU/g, vacuumed packaged, and stored at 8°C for 17 days or 3°C for 29 days. Both nisin and sodium lactate prevented the
growth of \textit{L. monocytogenes}. However, their use in combination with each other was more effective. The combination reduced numbers of the pathogen from 3.26 to 1.8 log CFU/g after 16 days when the trout was stored at 8°C. The levels of the pathogen remained constant between 4.66 and 4.92 log CFU/g for 29 days at 3°C.

The potential uses of sodium lactate as a replacement for the preservatives potassium sorbate and sodium benzoate in products such as comminuted sausages has also been studied (Choi and Chin, 2003). In that study, the researchers formulated sausages with 3.3% sodium lactate and compared them to a control, 0.05% or 0.1% potassium sorbate and sodium benzoate formulated sausages. The sausages were observed for how the preservatives influenced chemical composition, physico-chemical and textural properties, and growth of \textit{L. monocytogenes} when stored at 4°C for eight weeks. Sausages formulated with sodium lactate had lower thiobarbituric acid reacting substances values when compared to the control and potassium sorbate, varied lightness values, and texture was reduced after week 2 and week 6. Sodium lactate displayed similar antilisterial effects to the potassium sorbate or/and sodium benzoate and delayed the lag phase for the growth of \textit{L. monocytogenes} by two weeks compared to the control.

Malicki et al. (2004) evaluated 2% sodium lactate used alone or in combination with 200 ppm of lysozyme on microbial counts and physico-chemical properties in steamed sausages. Lactate used alone or with lysozyme performed better than the controls. Both worked synergistically against lactic acid bacteria in the sausages, but this synergism was not observed using total plate count. Sodium lactate and lysozyme had no negative effect on organoleptic and physico-chemical properties of the meat product either.
Apart from being used to control *L. monocytogenes*, sodium lactate has been used to control other organisms as well. Along with *L. monocytogenes*, *Yersinia enterolitica* had its lag phase extended by the combination of sodium lactate and ALTA 2341 (Barakat and Harris, 1999). Sodium lactate, along with sodium nitrite and sodium chloride, enhanced the properties of the bacteriocin AS-48 to inhibit enterotoxin production of the LWL1 strain of *Bacillus cereus* (Abriouel et al., 2002).

The use of sodium lactate as an antimicrobial is not always favorable. In a study done by Murphy et al (2004), the addition of sodium lactate increased the heat resistance of *L. monocytogenes* in certain meat products. The *D*-value for the pathogen in ground chicken thigh and leg meat, with a sodium lactate concentration of 4.8% was 53 to 75% higher than meat which had no sodium lactate.

**Regulatory status:**

The maximum level for sodium lactate in ready-to-eat meats is 4.8% of 60% w/v solution which is commercially available (Code of Federal Regulations, 1991).

**Sodium diacetate**  \( \text{C}_4\text{H}_7\text{O}_4\text{Na}\cdot\text{xH}_2\text{O} \)

**Regulatory status and general characteristics:**

In recent years, sodium diacetate has been approved for use as an antimicrobial in meat products. The permissible level for sodium diacetate in meat products is 0.25% (Code of Federal Regulations, 1991). One of the adverse effects of the addition of sodium diacetate to meat products is a vinegar-type flavor. This is most likely due to the presence of two acetate ions for each sodium ion (Miller, 2005). Numerous studies have been conducted to evaluate the antimicrobial effects of this organic acid salt in meat products.
Characteristics in meat products:

Seman et al (2002) tested sodium diacetate (0-2%) along with sodium chloride (0.8-3.6%), potassium lactate syrup (0.25-9.25%) and moisture content of the finished product (45.5-83.5%) against the growth rate of \textit{L. monocytogenes} in cured ready-to-eat meats. Sodium diacetate ($P < 0.11$) and potassium lactate ($P < 0.001$) caused significant reductions in growth rate of \textit{L. monocytogenes}. Moisture significantly ($P < 0.11$) increased the growth rate of the pathogen while the effect of sodium chloride on growth was not statistically significant. In a study looking at generally recognized as safe (GRAS) preservatives, sodium lactate along with three others (sodium benzoate, sodium propionate, and potassium sorbate) were used as dipping solutions for turkey frankfurters at the different concentrations (15, 20, and 25 $\%$ w/v) to see how they would affect the growth of \textit{L. monocytogenes}. The frankfurters were inoculated after dipping and kept in sterile stomacher bags where they were held at 4, 13, and 22°C for 14 days. All of the treated frankfurters contained \textit{L. monocytogenes} that were 3 to 4 logs fewer than those that were untreated after 14 days. Even under temperature abuse 25% sodium diacetate and sodium benzoate inhibited the organism’s growth seven days long than untreated franks (Islam et al., 2002). Yoon et al (2004) went further by not only applying a combination of sodium diacetate and potassium lactate to smoked salmon, but evaluated the effect of prior freezing on growth and survival of \textit{L. monocytogenes} Scott A. The adding of the potassium lactate and sodium diacetate combination reduced the survivors of \textit{L. monocytogenes} Scott A. Freezing significantly extended the lag phase and delayed growth of the organism at both 4 and 10°C.

Sodium diacetate is used as an antimicrobial agent, but there have been numerous times when it has been added in combination with other antimicrobials such as sodium
lactate (Bedie et al., 2001; Mbandi and Shelef, 2001; Mbandi and Shelef, 2002; and Uhart et al., 2004; Lu et al., 2005; Lungu and Johnson, 2005). Another example of that is stated in the previous paragraph. There was one instance where the combination of the two organic acid salts were bacteriocidal for a single strain and bacteriostatic for a six strain mixture of *L. monocytogenes* in bologna stored at 5°C (Mbandi and Shelef, 2002).

Researchers have conducted studies where sodium diacetate was tested in comparison to other organic acid salts, but not in combination. In cured ham products formulated with 0.2% sodium diacetate, the sodium diacetate had a negative effect on odor and taste. Sodium diacetate did inhibit the growth of *L. monocytogenes* as did sodium lactate (2.5-3.3%), but it did not inhibit *L. curvatus* (Stekelenburg and Kant-Muermans, 2001). Diacetate (0.25%) also reduced the growth of *Clostridium perfringens* in roast beef, but in another study it did not inhibit growth of the pathogen in cooked vacuum-packaged restructured roast beef for an 18 hour period (Sabah, 2003).

**Liquid Smoke**

**Background Information**

Liquid smoke is used as an artificial flavoring which is currently used in comminuted meat products. It is formulated from wood smoke condensate and water, and the filtered to remove polararomatic hydrocarbon containing materials which are carcinogenic (Pszczola, 1995). Not only is liquid smoke used for flavor purposes, but it contains high-boiling phenols which display bactericidal properties (Radecki et al., 1975).
Studies in Meats and Fish

Eklund et al. (1982) showed that liquid smoke used in combination with sodium chloride was effective as an inhibitor of growth and toxin production of *Clostridium botulinum* types A and E in hot-processed fish under temperature abuse at 25°C for 7 or 14 days. When the fish were inoculated on the surface with *C. botulinum* type E, there was toxin production in control samples with 3.7% water-phase NaCl but not in those treated with liquid smoke having 2.0% water-phase NaCl. However, the liquid smoke treatment was less effective when type E spores were injected into the fish. Finally, liquid smoke lowered the NaCl concentration requirement for inhibiting *C. botulinum* type A toxin production from 4.6 to 2.8% for those samples stored for days.

There have also been several studies involving liquid smoke and its inhibitory properties on *Listeria monocytogenes*. Before there were any reported outbreaks of listeriosis in the United States due to meat consumption, Messina et al. (1988) tested five smoke products for their antimicrobial properties against *L. monocytogenes* LCDC 81-861. The liquid smoke products were CharSol-10, Aro-Smoke P-50, CharDex, CharSol PN-9, and CharOil Hickory in concentrations of 0.5 and 0.25%. CharSol-10, Aro-Smoke P-50, CharDex at 0.5% lowered the viable cell counts below detection within four hours while CharSol PN-9 and CharOil Hickory did the same within 24 hours. In those samples with 0.25%, CharSol-10 and Aro-Smoke P-50 had similar effects in four hours, while CharDex, CharSol PN-9, and CharOil Hickory exhibited lowered antimicrobial effects at that concentration and required 24, 48, and 96 hours respectively to decrease microbial load below detection. CharSol-10 was selected as a full strength dip for beef franks because it showed strong antimicrobial
effects *L. monocytogenes* in pure culture. After being vacuum packaged and stored at 4°C for 72 hours, CharSol-10 displayed more that 99.9% reduction of *L. monocytogenes*.

In a related study, the growth behavior of *L. monocytogenes* was observed in wiener exudate or Tryptose Broth in the presence of liquid smoke and smoke ingredients, specifically isoeugenol (Faith et al., 1992). In untreated wiener exudate, the pathogen grew to 2.7 log CFU/ml when held at 25°C for 114 hours in the control sample. On the other hand, the exudates treated with 0.2 and 0.6% liquid smoke decreased numbers of *L. monocytogenes* with D-values of 36 hours for 0.2% and 4.5 hours for 0.6%.

Another study investigated at heat interaction and liquid smoke or generated smoke inactivating *L. monocytogenes* in hot-smoked salmon (Poysky et al., 1997). The minimum temperature needed to inactivate the organism was 153°F (67.2°C) when generated smoke was applied throughout the process. CharSol C-10, administered as a 100% dip before processing, inactivated *L. monocytogenes* in samples processed at temperatures as low as 138°F (58.9°C). When it was applied as a 50% concentration, the temperature lethality increased to a range of 145 to 150°F (62.8 to 65.6°C). When it was diluted even further to 25 and 10%, the inactivating temperature rose to 156°F (68.9°C) and 163°F (72.8°C) respectively. The researchers concluded that heat alone was not enough the inactivate *L. monocytogenes*.

Thurette et al. (1998) performed a study to determine how *L. monocytogenes* would react to liquid smoke, NaCl, and temperature in a laboratory medium and in fresh products. The concentration of liquid smoke was expressed as phenol concentration and it turned out that 20 ppm of the phenol concentration in combination with 4% NaCl was effective in
inhibiting the growth of \textit{L. monocytogenes} in synthetic laboratory medium. The results were similar in smoked fish products inoculated with the bacterium, and the deviation among the observed and predicted values was with 0.5 log units.

Liquid smoke has been shown to be a potent inhibitor of \textit{L. monocytogenes}. One study showed that concentrations of 60 to 100\% reduced \textit{Listeria innocua} by 3 logs CFU/g (Vitt et al., 2001). The reason \textit{L. innocua} was used was because it can survive in similar conditions to \textit{L. monocytogenes} which was indicated by \textit{in vitro} assays.

\section*{Sodium Lauryl Sulfate}

\begin{center}
\textbf{C}_{12}\text{H}_{25}\text{NaO}_{4}\text{S}
\end{center}

\textbf{Uses and Mechanism of Action}

Sodium lauryl sulfate (SLS) is one of many anionic surfactants used to sanitize the contact surfaces of food and is characterized by a balance between a hydrophobic residue and negatively charged hydrophilic group (Dychdala, 1983). The mechanism of action of anionic surfactants seems to involve denaturation of cell proteins, enzyme inactivation, and disruption of cell membranes and permeability (Dychdala, 1983). A point of interest is that anionic surfactants such as SLS are more effective against gram-positive and less on gram-negative bacteria (Cowles, 1938; and Baker et al., 1941).

\section*{Studies in Food}

Research data on the antimicrobial effects of SLS on foods, especially meat products, is scarce. However, there was one study that demonstrated the inhibitory effect of SLS against \textit{Staphylococcus aureus}. Buffered organic acid anionic surfactant (BOAAS), which is basically a combination of SLS, citric acid, and ethylenediaminetetraacetic acid (EDTA), was
shown to reduce *Staphylococcus aureus* significantly at concentrations ranging from 0.6 to 1.75% in a laboratory medium (Restaino et al., 1994). There was also a particular study where SLS did not perform as well. SLS removed 1.5 log CFU/ml of *Salmonella* from strawberries and lettuce when administered as a 0.1% rinse, but was no more effective than Tween 80 at the same concentration and water (Raiden et al., 2003).

To our knowledge, the only published research that utilized SLS with meat to control pathogens was performed by Tamblyn and Conner (1997). The purpose for that study was to determine if certain treatments were bactericidal against *S. typhimurium*. When applied alone, 125 ppm of SLS was not bactericidal. But, once it was combined with lactic acid it reduced the organism on chicken skin by 1.29 log. This was dependent however on the acid concentration and the means of application.

**Food Irradiation**

**Brief History**

W. C. Roentgen discovered X-rays in 1895 and A. H. Becquerel observed radiation from uranium the following year. These can be considered two of the first moments in the history of irradiation. The first documented application of food irradiation in food was in 1921 when B. Schwartz of the U.S. Department of Agriculture (USDA) proposed the use of X-rays to inactivate trichinae in pork. More interest came when A. Brasch and W. Huber invented a pulsed electron accelerator in which food could be sterilized from high energy electron pulses. During the 1950s-1960s, the U.S. Army conducted research using low-dose and high dose irradiation of military rations (Josephson, 1983 and Diehl, 1995).
Requirements for Application to Meats

For meats and poultry, approval for the use of irradiation is required by both the Food and Drug Administration (FDA) and the USDA. The FDA requires a limit of no more than 3.0 kGy in poultry and the USDA has range from 1.5-4.5 kGy. Both federal entities have a maximum limit of 4.5 kGy for fresh meats and 7.0 kGy for frozen meats (Tauxe, 2001). To date the use of ionizing irradiation on ready-to-eat processed meats has not been approved.

Types of Irradiation

There are four types of irradiation used in controlling microbial growth of foods. Those radiation types are gamma rays, electron beams, X-rays, and ultraviolet light (UV). The reason that these radiation types are called ionizing irradiation is because molecular ions form when electrons interact with matter (Jay, 2000; Tauxe, 2001; IAEA, 2002; and Morehouse and Komolprasert, 2004). Gamma rays are emitted by Cobalt-60 or Cesium-137 radioisotopes. Gamma irradiation is referred to as being monoenergetic, meaning that the radioisotope emits simultaneously two photons per disintegration with energy levels of 1.17 and 1.33 MeV. Two-sided irradiation can be achieved by turning the product or irradiating the product from two sides of a source.

Electron-beam irradiation is applied when electrons are emitted by accelerators with an energy as high as 10 MeV. If a product is 9 cm thick, it can be treated with 10 MeV electrons. However, if a food product is much thicker, only a limited amount of sub-surface treatment will be achieved.

The application of X-rays is similar to that of electron-beam irradiation. It is achieved by emitting an electron beam over a selected target. The only difference is that it can only go to an energy level as high as 5 MeV. Unlike gamma irradiation, the sources emit photons
with a broad spectrum of energy (IAEA, 2002). UV irradiation is not used as frequently as the previous types.

**Mechanism of Antimicrobial Action**

Ionizing irradiation causes DNA strand breaks, transition mutations, frameshift mutations, and deletions. Ionizing irradiation can also disrupt the cell membrane associated with DNA complexes required for plasmid portioning and sites which are active for DNA repair and cause loss of plasmids carrying genes for pathogen virulence (Sommers et al., 2004). An irradiation dose of 2.0 to 2.5 kGy is needed for a 1 to 2% single gene inactivation rate (Sommers et al., 2004). As it relates to *L. monocytogenes*, disruption of the cell membrane can lead to increased sensitivity organic acids and other antimicrobial compounds such as sodium diacetate (Sommers and Fan, 2003).

**Factors that affect the Antimicrobial Action of Irradiation**

According to Jay (2000) there are several underlying factors that will determine how effective irradiation will be on foods regarding antimicrobial activity. One factor is the types of organisms present. Gram-positive bacteria are more resistant than gram-negatives to irradiation. Also, spore formers are more resistant that non-spore formers. The number of organisms present is another major factor on antimicrobial action. The larger the cell population that is present, there will be a decrease in the effectiveness of a given dose.

Another factor influencing the antimicrobial action of irradiation is that microorganisms are more susceptible to irradiation when suspended in buffer solutions than in those containing protein. The reason for this is that proteins act as a protective covering from radiation by shielding microorganisms from free radicals. Oxygen presence is another
key factor. If oxygen is not present in food product, the microorganisms will be more resistant to irradiation due to reduced formation of toxic oxygen derivatives and free radicals.

Other factors influencing irradiation effectiveness are the physical state of the food and the age of the organisms. Dried cells have a higher radiation resistance than moist cells, due to decreased production of free radicals resulting from radiolysis of water. With regard to age of cells, those in lag phase are more resistant before they begin actively dividing. Cells become more sensitive once they enter log phase and stationary phase cells are very resistant to radiation.

Finally, temperature can also have an impact on the effectiveness of irradiation. Microorganisms are more easily inactivated when they are at temperatures above freezing. When there is decreased water activity due to freezing of water, free radicals cannot move freely thereby decreasing the chances for radiolysis to occur (Diehl, 1995).

**Control of Pathogens in Meats**

There have been several studies by which researchers observed the effects of irradiation for controlling *L. monocytogenes* (Patterson et al., 1993; Sommers and Fan, 2003; and Foong et al., 2004). Patterson et al. (1993) observed the effects of irradiation and temperature on the fate of *L. monocytogenes* in poultry meat (raw and cooked). They found that the use of an irradiation dose of 2.5 kGy and a storage temperature of 6°C which extended the lag phase of the pathogen. This showed that the higher the irradiation dose and the lower the storage temperature, the longer will be the lag phase.

There is relatively few published research on the effects of irradiation for controlling pathogens in ready-to-eat meats. Foong et al. (2004) demonstrated 3 and 5-log reductions of *L. monocytogenes* in a variety of ready-to-eat meats through electron beam irradiation. The
dose required for 3 and 5-log reductions were 1.5 and 2.5 kGy respectively. The average D-value for the bacterium was 0.44 kGy. *L. monocytogenes* had decreased survival when irradiated at 4 kGy and stored at 4° and 10°C for 12 weeks.

Sometimes the application of irradiation is not enough to control the growth of microorganisms at doses that do not negatively affect meat quality. In such instances the combined use of irradiation and other antimicrobial interventions is necessary. Sommers and Fan (2003) found that using irradiation and sodium diacetate had a synergistic effect in controlling the growth of *L. monocytogenes* in fine-emulsion sausage. They were able to achieve reductions ranging from 2.5 to 5 log CFU/g for irradiation doses 1.5 to 3 kGy respectively. The addition of sodium diacetate increased the bacteriostatic effects.

Not only has irradiation been used to control *L. monocytogenes*, but other pathogens as well such as *Escherichia coli* O157:H7 (Arthur et al., 2005). Arthur et al. (2005) found in their study that 1.0 kGy can reduce this pathogen by at least 4 log CFU/cm² on sections of cutaneous trunci of beef carcasses. Besides *L. monocytogenes* and *E. coli* O157:H7, *Campylobacter jejuni* and *Salmonella* can be destroyed by irradiation (Tauxe, 2001) which can be used to extended shelf life of products such as pork loins (Kim et al., 2004).
REFERENCES


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CHAPTER 2. FATE OF LISTERIA MONOCYTOGENES FOLLOWING ELECTRON-BEAM IRRADIATION OF VACUUM-PACKAGED FRANKFURTERS FORMULATED WITH SODIUM LACTATE AND SODIUM DIACETATE AND STORED AT 4° AND 10°C

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Lawrence Cobb1, Aubrey Mendonca1, James Dickson2, and Joseph Sebranek1,2

1Department of Food Science and Human Nutrition and 2Department of Animal Science, Iowa State University, Ames, Iowa 50011

ABSTRACT

A study was conducted to determine the fate of Listeria monocytogenes following irradiation of vacuum-packaged frankfurters formulated with 2 or 3% (w/w) sodium lactate (SL), 0.1% (w/w) sodium diacetate (SDA), or SL (2 or 3%) + SDA (0.1%). Samples of the product without SL or SDA served as controls. Frankfurters inoculated with L. monocytogenes (10⁶ CFU/frankfurter) were vacuum-packaged, irradiated (0.0, 1.0, and 2.0 kGy), and stored at 4°C for 90 days or 10°C for 36 days. At set time intervals, each frankfurter was washed with 20 ml of sterile 0.1% (w/v) peptone water and 0.1-ml aliquots of wash solution were surface-plated onto Modified Oxford (MOX) agar. Colonies of L. monocytogenes survivors on MOX agar plates were counted at 48 hrs after incubation (35°C). L. monocytogenes on control frankfurters grew rapidly and reached greater than 9.0 log CFU/frankfurter at day 60 (4°C) and day 18 (10°C). Irradiation of frankfurters at 1.0 and 2.0 kGy reduced initial numbers of the pathogen by ~1.5 to 1.8 log and 2.7 to 3.2 log, respectively. Irradiation (2.0 kGy) extended the lag phase of L. monocytogenes in refrigerated (4°C) controls to 60 days. In contrast, growth of the pathogen was completely inhibited in frankfurters containing 2% or 3% SL irrespective of irradiation dose or storage temperature. Apart from SDA that reduced
the initial pH of frankfurters by 0.15 units, other antimicrobial treatments did not affect pH. Based on the results of this study, a combination of electron-beam irradiation (2.0 kGy) and SL (2 or 3% in frankfurter formulation) has good potential for reducing initial numbers of *L. monocytogenes* and preventing growth of survivors in frankfurters during refrigeration or temperature abuse. Further studies are needed to optimize dose levels of irradiation and concentrations of SL in frankfurter formulation to ensure microbial safety of frankfurters while maintaining the sensory attributes of this popular ready-to-eat meat product.

**INTRODUCTION**

*Listeria monocytogenes* is a gram-positive, facultatively anaerobic, non-sporeforming, pathogenic, rod-shaped bacterium that is a major food safety concern in ready-to-eat (RTE) deli-style meat products. This organism is psychrotrophic and grows within a temperature range of 0°C to 45°C with an optimum growth temperature at 37°C. It can also grow at pH levels between 4.4 and 9.4 and at a water activity greater than 0.92. *L. monocytogenes* is ubiquitous in the environment and has been isolated from sources such as soil, vegetation, silage, fecal material, sewage, and water (24). It has also been isolated in foods such as raw and pasteurized milk, cheeses, ice cream, raw vegetables, raw and cooked meats. Besides being present in raw foods of plant or animal origin, *L. monocytogenes* may also be present in RTE foods because of post-processing contamination.

*L. monocytogenes* causes listeriosis, an invasive disease that can be life threatening to susceptible populations. Among those hospitalized, the fatality rate is between 20 to 30%. An infective dose of the pathogen is $10^3$ CFU/ml or g, but this can vary with the immune status of individual (9, 24). *L. monocytogenes* serotype 4b strains have been linked to several well-
publicized listeriosis outbreaks in the United States (14). Many of these outbreaks involved the consumption of RTE meat products. For example in 1998 frankfurters distributed by a processing plant in Michigan were blamed for causing 101 cases of listeriosis and 20 deaths (5). In 2002, two separate outbreaks were associated with consumption of sliced RTE turkey meat products (6, 8). In the first outbreak 16 of 44 persons who attended a catered event suffered from a fever and acute gastroenteritis after eating contaminated deli meat (12). The second outbreak was associated with 46 culture-confirmed cases, 7 deaths and 3 stillbirths (6). Because of the high mortality rate of listeriosis, the USDA’s Food Safety Inspection Service (FSIS) has enforced a zero tolerance policy for L. monocytogenes in ready-to-eat (RTE) meat products (9).

One strategy for destroying L. monocytogenes in RTE meats is through the application ionizing irradiation. The most common food irradiation techniques involve the application of gamma rays, X-rays, or electron beams (16). The one applied in this study was electron beam irradiation. Electron beam irradiation has been evaluated and shown to be a prime intervention for destroying harmful organisms like Escherichia coli O157:H7 in food products (1, 28) and extend shelf life of foods (15). Arthur et al. (1) showed that a low dose of electron beam irradiation, 1 kGy, reduced E. coli O157:H7 inoculated on a beef carcass by at least 4 log CFU/cm². Along with reducing microbial loads in fresh foods, ionizing irradiation within the dose range of 0.7 to 4.5 kGy has been shown to reduce microflora in ready-to-eat food products (10, 11, 27).

Another method for controlling L. monocytogenes is the incorporation of antimicrobials into the formulation of RTE meats, specifically organic acids. Two organic acids in particular that have been well studied are sodium lactate (SL) and sodium diacetate
(SDA). Studies have shown that both compounds have inhibitory properties to the organism (2, 3, 4, 17, 18, 20). Other studies have also shown that SL and SDA were used to effectively control other organisms such as Clostridium perfringens, Yersinia enterolitica, Salmonella Enteritidis, and lactic acid bacteria (2, 19, 20, 25). However, some of these studies used maximum permitted levels of organic acid salts or levels that exceeded the maximum allowed by the federal government in RTE meats. Permissible levels for SL and SDA are 4.8% and 0.25% respectively (7). Bedie et al. (4) evaluated levels of 0.25% SDA and 3% SL in frankfurters which was inhibitory to L. monocytogenes. They also evaluated levels of 0.5% SDA which retarded the growth the pathogen by 1-2 logs and 6% SL whose inhibitory effects lasted 90 days as opposed to 50 day when 3% SL was used.

There is a rapidly increasing amount of published information on the antilisterial effects of sodium lactate and sodium diacetate added to RTE meats. However, published research on the fate of L. monocytogenes following electron-beam irradiation in frankfurters formulated with lactate and/or diacetate is scarce. Accordingly, the objective of this study was to use a combination of irradiation treatments and organic acids salts (sodium lactate and sodium diacetate) incorporated into the formulation of frankfurters to control the growth of L. monocytogenes in this product.

MATERIALS AND METHODS

Preparation of frankfurters. Frankfurters were prepared at the Iowa State University Meat Laboratory using meat trimmings that consisted of boneless beef (80:20 lean: fat ratio) and pork fat (20:80 lean: fat ratio). Both of the meat types were ground separately using a 0.79-cm grinder plate. The mixture of the beef trim, salt, sodium erythorbate, sodium nitrite,
seasoning, and half the total water (added as ice) were chopped for about 3 min in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA). The basic formulation was used for preparing control frankfurters whereas separate batches of this formulation were mixed with sodium lactate (SL) and/or sodium diacetate (SDA) to prepare meat batter with the following final concentrations of added organic salts on a weight basis: 2% SL, 3% SL, 0.1% SDA, 2% SL + 0.1% SDA, or 3% SL + 0.1% SDA. When the temperature of the mixture reached 4.4°C (40°F) the pork trim, pork fat and the remainder of the ice was added. This mixture was chopped for 3 min until the temperature of the meat batter reached about 12°C (53.6°F). The meat batter was stuffed into 22-mm diameter peelable cellulose casings (Devro TeepackWiener-Pak, Coastal Corrugated Inc., North Charleston, SC). The encased product was linked at 14 cm length x 2.2 cm diameter using a Poly-clip System (Kramer & Grebe GMBH & Co., Frankfurt, Germany).

The linked product was placed on racks and cooked in a humidity controlled smoke house (Alkar, DEC Intl. Inc., Lodi, WI) using a cooking-smoking cycle for 90 min. The final internal temperature of the cooked product was 71.1°C. The frankfurters were showered with cool water and chilled at 4°C for approximately 18-19 h. The next day, frankfurters were peeled with a peeler (Townsend Engineering Co., Des Moines, IA). The frankfurters were then vacuum-packed in a Multivac 46800 (Multivac Sepp Haggenmuller, Gmblt & Co., Wolfertschwenden, Germany) and stored at -20°C in a walk-in freezer until used in experiments.

**Bacterial strains and preparation of inoculum.** 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.

From each individual *L. monocytogenes* working culture, 0.1 ml was aseptically transferred to 10 ml of Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE) (Difco) and incubated at 35°C for 18 h. A second transfer was performed by transferring 1 ml from each individual tube to separate flasks each containing 100 ml of TSBYE (Difco). The cultures were incubated at 35°C for 18 h. A 5-strain mixture of *L. monocytogenes* was prepared when 6 ml of each culture were combined to yield a 30 ml mixture in a sterile 50-ml centrifuge tube. The cells were harvested by centrifugation (10,000 x g for 10 min at 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Wilmington, DE). The supernatant was discarded and the pelleted cells were resuspended in 0.1% peptone water (Difco) and washed once by centrifugation (10,000 x g, 10 min, 4°C). Pellets of washed cells were resuspended in 30 ml of fresh 0.1% peptone water to give a final cell concentration of approximately $10^9$ colony forming units (CFU) per ml. This washed cell suspension was used to inoculate the frankfurters.

**Inoculation of samples.** Ten-fold serial dilutions of washed cell suspension were performed using 0.1% peptone. Frankfurters were placed in vacuum-packaging bags (Cyrovac B-2540, Cryovac Sealed Air Corp., Duncan, SC) and each inoculated with 0.1 ml of the appropriate dilution of washed cells to give a final concentration of $10^6$ cells/frankfurter. The packaged
franks were manually massaged for 10 s to spread the inoculum over the surface of each frank. Packages containing inoculated and non-inoculated franks were packaged under vacuum in a Multivac A 300/51 vacuum packaging machine (Multivac Sepp Haggenmuller, Gmbht & Co., Wolfertschwenden, Germany). All vacuum-packaged samples were held overnight (18-20 h) in a walk-in refrigerator (2-4°C) before subjected to irradiation

**Electron-beam irradiation and dosimetry.** One day after inoculation, the samples were transported in a cooler containing crushed ice to the irradiation facility at Iowa State University Meat Laboratory. The inoculated and non-inoculated samples (4°C) were irradiated with 0 (control), 1, and 2 kGy. The samples were irradiated with a MeV CIRCE III Linear Electron Accelerator (MeV Industries S. A., Jouyen-Josas, France) operating at energy level of 10 MeV and an average dose rate of 62.2 kGy/min. The absorbed doses were measured by use of 5 mm (diameter) by 5 mm (length) alanine pellets (Bruker Analytische Messtechnik, Rheinstetten, Germany) that were positioned on the top and bottom sides of selected packages of frankfurters. Immediately after the irradiation process, the absorbed doses were measured by placing the alanine pellets in a Bruker EMS 104 EPR Analyzer (Bruker Analytische Messtechnik, Rheinstetten, Germany) which uses electronic paramagnetic resonance to achieve this measurement.

**Microbial analysis.** The samples were assigned to two storage temperatures, 4°C and 10°C. Those stored at 4°C were analyzed for *L. monocytogenes* survivors on days 0, 14, 28, 42, 60 and 90, while those stored at 10°C were analyzed on days 0, 6, 12, 18, 24, and 36. Samples were pulled at random for microbial analysis. Vacuum-packaged bags containing inoculated franks were aseptically opened and 20 ml of sterile peptone water was added to each package
(13). The packages were massaged vigorously by hand for 30 s and 1-ml aliquots of the wash solution were serially diluted in 0.1% peptone water. Aliquots (0.1 ml) of appropriate dilutions were surface-plated on Modified Oxford Medium (MOX) (Difco) agar plates. All inoculated agar plates were then incubated aerobically at 35°C for 48 h. The plates were removed from incubation after 48 h and colonies were enumerated using a Leica 3325 Quebec Darkfield Colony Counter (Leica, Buffalo, NY). Plates having 25 to 250 typical \textit{L. monocytogenes} colonies were counted, and counts were expressed as log CFU/link.

**Measurement of pH.** The pH of irradiated, non-inoculated frankfurters stored at 4°C was evaluated at days 0, 14, 28, 60, and 90. Packages of frankfurters were opened and 300 ml of distilled water were added to each package. The mixture was pummeled in a Seward Stomacher 400 laboratory blender (Seward Ltd., London England) for 30 s at medium speed. The pH of the resulting slurry was analyzed using an Orion 420Aplus Benchtop pH meter (Thermo Electron Corp., Beverly, MA) fitted with a glass electrode.

**Statistical analysis.** Three independent replicate experiments were conducted and two frankfurter samples per treatment were analyzed for each replication of the experiment. Analysis of variance (ANOVA) was performed with the General Linear Models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC). Differences were considered statistically significant at $P<0.05$ unless otherwise stated. Differences among variables were tested for significance using Tukey’s honestly significant different multiple comparison test. Mean values and standard error of the means (SEM) were reported.
RESULTS AND DISCUSSION

Tables 2.1, 2.2 and 2.3 show the pH of frankfurters treated with 0.0, 1.0, and 2.0 kGy, respectively, and stored at 4°C for 90 days. Initially (day 0), SDA alone or combined with SL decreased the pH of non-irradiated frankfurters; pH decreases ranged from 0.04 to 0.15 units (Table 2.1). Although meat proteins provide good buffering capacity, the decrease in pH of the non-irradiated frankfurters observed in the present study is not surprising because SDA is a relatively strong acidulant. Bedie et al (4) reported that the initial pH of frankfurters formulated with 0.5% (w/w) sodium diacetate decreased by approximately 0.4 units. During storage, the pH of control decreased from 6.13 (day 0) to 5.96 (day 90). While no added organic acid salts were present in the control, the prolific growth of bacteria was the likely contributory factor to this pH decrease. There were no significant differences in pH among treatments during storage of irradiated frankfurters (Tables 2.1, 2.2 and 2.3) indicating less acid production due to the reduction of bacterial numbers by irradiation.

Populations of L. monocytogenes in non-irradiated (0.0 kGy) vacuum-packaged frankfurters at set time intervals during storage at 4 and 10 °C are shown in Figures 2.1 and 2.2. Initial numbers of L. monocytogenes were 10^6 CFU per frankfurter. This level of inoculation was used to facilitate the detection of L. monocytogenes survivors following irradiation treatment of the frankfurters. At 4 °C growth of the pathogen in control frankfurters occurred after 14 days and reached approximately 8.63 and 9.75 log CFU at 42 and 90 days, respectively. In frankfurters formulated with 0.1% SDA alone, the lag phase of growth was extended to 28 days and numbers of the pathogen increased slowly to reach 6.80 log CFU at 90 days. Growth of L. monocytogenes did not occur for 90 days in frankfurters formulated with SL (2 or 3%) alone or combined with 0.1% SDA.
These results are consistent with those of previous research (21, 23, 26) that reported the growth inhibitory effect of sodium or potassium lactate combined with sodium diacetate against *L. monocytogenes* when these organic acid salts are used in the formulation of cured meat products. Specifically, Porto et al. (23) observed that there was no growth of *L. monocytogenes* for 90 days in beef/pork frankfurters (4°C) formulated with 3% (w/w) potassium lactate. A combination of 2.5% (w/w) SL and 0.2% (w/w) SDA completely inhibited growth of a 6-strain mixture of *L. monocytogenes* in beef bologna (23). Samelis et al. (26) reported that a combination of 3% (w/w) SL and 0.25% (w/w) SDA in pork frankfurters prevented growth of a 10-strain mixture of *L. monocytogenes* for 120 days during storage of the product at 4°C.

Other studies have demonstrated the growth inhibitory effect of organic acid salts against *L. monocytogenes* when used singly in cured meat products for various time periods during refrigerated storage. For example, 3% SL was required to prevent the growth of *L. monocytogenes* in pork liver sausage (5°C) for 50 days (29). Neither 2% SL nor 0.5% sodium acetate prevented growth of *L. monocytogenes* in vacuum-packaged turkey bologna (4°C) after 70 to 90 days (31). Bedie et al (4) reported that 3% SL in pork frankfurters inhibited the growth of *L. monocytogenes* for 70 days during storage of this meat product at 4°C. In the present study and those of others (23, 26) a combination of SL and SDA completely suppressed growth of *L. monocytogenes* in refrigerated frankfurters for at least 90 days. This is important considering that the targeted commercial shelf-life of refrigerated cooked meat in the United States is 75 to 90 days (30). In this regard, combinations of antimicrobials added to the formulation of processed meat products should be able to prevent growth of *L. monocytogenes* for at least 90 days.
At 10 °C growth of *L. monocytogenes* occurred rapidly in control frankfurters and frankfurters formulated with 0.1% SDA alone (Figure 2.2). As early as 18 days numbers of the pathogen in control and frankfurters with 0.1% SDA reached 9.12 and 7.65 log CFU, respectively. Growth of the pathogen was completely inhibited for 36 days in frankfurters formulated with SL (2 or 3%) alone or combined with 0.1% SDA. The relatively faster growth of *L. monocytogenes* in frankfurters at 10 °C compared to 4 °C was not surprising because of the psychrotrophic characteristic of this organism. SL (3%) has been shown to prevent the growth of *L. monocytogenes* for 60 days in vacuum packaged frankfurters held at 10 °C (23). The use of 10 °C in the present study was intended to simulate temperature abuse than might occur in retail distribution of RTE meats. For example, prolonged delays in unloading RTE meats from refrigerated trucks at loading docks before stocking products in commercial refrigeration units is likely to cause increases in product temperature. Also, improperly functioning refrigeration units can expose meat products to temperatures above 2 to 4 °C that would increase the growth rate of *L. monocytogenes*. Based on the findings of the present study, 0.1% SDA alone is ineffective for preventing growth of *L. monocytogenes* at 10 °C whereas, growth in frankfurters at this abusive temperature can be prevented by SL (2 or 3%) alone or combined with 0.1% SDA.

The survival and growth of *L. monocytogenes* in irradiated (1.0 kGy) frankfurters stored at 4 and 10 °C are shown in Figures 2.3 and 2.4, respectively. Initial populations of the pathogen were reduced by about 1.50 log after irradiation. During storage survivors grew in control frankfurters and in those with 0.1% SDA alone irrespective of storage temperature. At 4 °C numbers of the pathogen increased to 7.52 and 9.54 log CFU, respectively, at 42 and 90 days. More rapid growth occurred at 10 °C compared to 4 °C; however, irradiation (1.0
kGy) delayed growth of the pathogen for 60 days (at 4 °C) and 12 days (at 10 °C) in frankfurters formulated with 0.1% SDA alone. At either temperature no growth of survivors occurred in frankfurters with 2 or 3% SL alone or combined with 0.1% SDA. In fact, frankfurters that contained these organic acid salts exhibited approximately 1.0-log decrease in numbers of survivors at 90 days (at 4 °C) and 36 days (at 10 °C) of storage.

The growth of *L. monocytogenes* survivors in control frankfurters following irradiation with 1.0 kGy indicates that unless irradiation doses are high enough to completely eliminate this pathogen in frankfurters, it will be necessary to have bacteriostatic levels of antimicrobials in the product to prevent its growth. In the present study, the incorporation of SL (2 or 3%) with or without 0.1% SDA in frankfurter formulation proved to be bacteriostatic to *L. monocytogenes* whether or not frankfurters were irradiated. Therefore, the application of low dose irradiation (≤1.0 kGy) to destroy initial populations of *L. monocytogenes* along with antimicrobials to prevent growth of survivors, if present, may be a good strategy for control of this pathogen in ready-to-eat meats. While irradiation of frankfurters that contained 0.1% SDA alone resulted in increased lag phase in growth of the pathogen at 10 °C, the lag phase at 4 °C was about 48 days longer. This result is most likely due to irradiation injury of the pathogen and a relatively longer time required by the organism to repair its lesions at lower temperatures (21). The 1.0-log decrease in *L. monocytogenes* survivors observed in irradiated frankfurters at 90 days (at 4 °C) and 36 days (at 10 °C) of storage might be attributed to a sub-population of injured cells that failed to repair irradiation-induced injury and therefore were not able to grow on MOX agar.

Populations of *L. monocytogenes* survivors in irradiated (2.0 kGy) frankfurters stored at 4 and 10 °C are shown in Figures 2.5 and 2.6, respectively. Reductions in the initial
population of \textit{L. monocytogenes} ranged from \~{}2.89 to 3.32 log CFU depending on product formulation. These results are consistent with those reported by Foong et al. (2004) who demonstrated a 3.0 log reduction of a 5-strain cocktail of \textit{L. monocytogenes} in vacuum packaged frankfurters (4 °C) following irradiation with 2.0 kGy. In the present study, reductions of initial population of the pathogen in frankfurters formulated with organic salts were consistently greater than in controls; however, differences were not significant (P>0.05). At 4 °C no growth of \textit{L. monocytogenes} survivors was observed in control frankfurters until after 60 days when numbers of the pathogen increased to greater than 7.0 log CFU at 90 days. Also, no increase in numbers of survivors occurred for 90 days in frankfurters formulated with SL or SDA alone or in combination (Figure 2.5). In contrast, at 10 °C, survivors grew in the control with numbers increasing to 6.47 and 9.38 log CFU at 12 and 24 days, respectively (Figure 2.6). Growth of survivors in frankfurters formulated with 0.1\% SDA was only inhibited for 12 days at 10 °C and increased to 4.5 and 7.0 log CFU at 18 and 36 days, respectively. No growth of survivors was observed in frankfurters with SL (2 or 3\%) alone or combined with 0.1\% SDA (Figure 2.6). These results clearly indicate that temperature abuse of irradiated processed meats that might have \textit{L. monocytogenes} survivors in the absence of bacteriostatic formulation ingredients can lead to faster resuscitation and growth of the pathogen to compromise the microbial safety of these meat products. Also, a sub-bacteriostatic concentration of an antimicrobial such as 0.1\% SDA that just slows growth of the pathogen might not be effective in preventing resuscitation of cells sub-lethally injured by irradiation and subsequent growth of those cells upon repair of their injury. Alternatively, the use of bacteriostatic concentrations of antimicrobials in ready-to-eat meats can provide an
extra layer of microbial safety in these products following irradiation and during refrigerated storage or exposure to abusive temperatures.

Based on the findings of the present study, a combination of electron-beam irradiation (2.0 kGy) and SL (2 or 3% in the product formulation) has good potential for reducing initial populations of \textit{L. monocytogenes} and preventing growth of survivors in vacuum packaged frankfurters during refrigeration (4 °C) and temperature abuse (10 °C). There is an urgent need for additional studies to optimize irradiation dose and concentrations of antimicrobial formulation ingredients to ensure microbial safety of ready-to-eat meat products.

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**REFERENCES**


Table 2.1 *Mean pH values of non-irradiated (0 kGy) vacuum-packaged frankfurters formulated with or without sodium lactate and sodium diacetate and stored at 4°C*

<table>
<thead>
<tr>
<th>Product</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>6.13±0.17</td>
<td>5.95±0.41</td>
<td>6.04±0.03</td>
<td>5.96±0.07</td>
<td>5.96±0.14</td>
</tr>
<tr>
<td>2% SL</td>
<td>6.13±0.17</td>
<td>6.00±0.23</td>
<td>6.03±0.06</td>
<td>6.00±0.08</td>
<td>6.03±0.15</td>
</tr>
<tr>
<td>3% SL</td>
<td>6.09±0.20</td>
<td>5.98±0.19</td>
<td>6.01±0.04</td>
<td>6.03±0.12</td>
<td>6.03±0.14</td>
</tr>
<tr>
<td>0.1% SDA</td>
<td>5.94±0.19</td>
<td>5.86±0.23</td>
<td>5.80±0.01</td>
<td>5.84±0.13</td>
<td>5.86±0.13</td>
</tr>
<tr>
<td>2%SL + 0.1% SDA</td>
<td>6.03±0.13</td>
<td>5.93±0.17</td>
<td>5.82±0.01</td>
<td>5.83±0.12</td>
<td>5.85±0.15</td>
</tr>
<tr>
<td>3%SL + 0.1% SDA</td>
<td>5.98±0.10</td>
<td>5.94±0.16</td>
<td>5.81±0.01</td>
<td>5.84±0.14</td>
<td>5.84±0.15</td>
</tr>
</tbody>
</table>

*Means (standard deviations of three replicate experiments)
SL = (sodium lactate); SDA = (sodium diacetate)
Table 2.2: Mean pH values of irradiated (1 kGy) vacuum-packaged frankfurters formulated with or without sodium lactate and sodium diacetate and stored at 4°C

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.16±0.06</td>
<td>6.12±0.19</td>
<td>5.99±0.03</td>
<td>6.01±0.15</td>
<td>6.03±0.16</td>
</tr>
<tr>
<td>2% SL</td>
<td>6.17±0.07</td>
<td>6.07±0.20</td>
<td>5.99±0.01</td>
<td>6.05±0.20</td>
<td>6.02±0.16</td>
</tr>
<tr>
<td>3% SL</td>
<td>6.18±0.07</td>
<td>6.11±0.21</td>
<td>6.01±0.02</td>
<td>6.03±0.14</td>
<td>6.00±0.14</td>
</tr>
<tr>
<td>0.1% SDA</td>
<td>6.08±0.04</td>
<td>5.94±0.23</td>
<td>5.78±0.02</td>
<td>5.83±0.13</td>
<td>5.80±0.11</td>
</tr>
<tr>
<td>2%SL + 0.1% SDA</td>
<td>6.06±0.04</td>
<td>5.97±0.17</td>
<td>5.80±0.03</td>
<td>5.83±0.14</td>
<td>5.84±0.13</td>
</tr>
<tr>
<td>3%SL + 0.1% SDA</td>
<td>6.06±0.02</td>
<td>5.94±0.16</td>
<td>5.80±0.04</td>
<td>5.83±0.14</td>
<td>5.86±0.13</td>
</tr>
</tbody>
</table>

aMeans (standard deviations of three replicate experiments)
SL = (sodium lactate); SDA = (sodium diacetate)
Table 2.3 Mean pH values of irradiated (2 kGy) vacuum-packaged frankfurters formulated with or without sodium lactate and sodium diacetate and stored at 4°C

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 28</th>
<th>Day 60</th>
<th>Day 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.18±0.06</td>
<td>6.11±0.17</td>
<td>5.97±0.05</td>
<td>6.00±0.15</td>
<td>6.03±0.13</td>
</tr>
<tr>
<td>2% SL</td>
<td>6.17±0.09</td>
<td>6.11±0.14</td>
<td>5.99±0.02</td>
<td>6.01±0.16</td>
<td>6.03±0.11</td>
</tr>
<tr>
<td>3% SL</td>
<td>6.18±0.07</td>
<td>6.15±0.15</td>
<td>6.02±0.05</td>
<td>6.02±0.16</td>
<td>6.05±0.14</td>
</tr>
<tr>
<td>0.1% SDA</td>
<td>6.11±0.06</td>
<td>5.97±0.15</td>
<td>5.78±0.03</td>
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<td>6.09±0.05</td>
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<td>6.07±0.07</td>
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<td>5.84±0.15</td>
<td>5.85±0.13</td>
</tr>
</tbody>
</table>

aMeans (standard deviations of three replicate experiments)
SL = (sodium lactate); SDA = (sodium diacetate)
Figure 2.1 Populations of *Listeria monocytogenes* in non-irradiated (0 kGy) frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 4°C.
Figure 2.2 Populations of *Listeria monocytogenes* in non-irradiated (0 kGy) frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 10°C.
Figure 2.3 Populations of *Listeria monocytogenes* survivors following irradiation (1 kGy) in frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 4°C.
Figure 2.4 Populations of *Listeria monocytogenes* survivors following irradiation (1 kGy) in frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 10°C.
Figure 2.5 Populations of *Listeria monocytogenes* survivors following irradiation (2 kGy) in frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 4°C.
Figure 2.6 Populations of Listeria monocytogenes survivors following irradiation (2 kGy) in frankfurters formulated with or without sodium lactate (SL) and sodium diacetate (SDA) and stored at 10°C.
CHAPTER 3. COMBINED ANTIMICROBIAL ACTIVITY OF LIQUID SMOKE, SODIUM LAURYL SULFATE, AND ELECTRON-BEAM IRRADIATION AGAINST LISTERIA MONOCYTOGENES IN VACUUM PACKAGED FRANKFURTERS STORED AT 4°C

A paper submitted to Food Microbiology

Lawrence Cobb¹, Aubrey Mendonca¹, James Dickson², and Joseph Sebranek¹,²

¹Department of Food Science and Human Nutrition and ²Department of Animal Science, Iowa State University, Ames, Iowa 50011

ABSTRACT

This study evaluated the antimicrobial activity of liquid smoke, sodium lauryl sulfate, and electron-beam irradiation against Listeria monocytogenes in vacuum packaged frankfurters. Frankfurters formulated with or without 2% (w/w) sodium lactate (SL) were immersed (2 min) in distilled water (control), 30% (v/v) liquid smoke (LQS), 1% (w/v) sodium lauryl sulfate (SLS) or LQS + SLS then drained for 30 seconds. Samples were inoculated with a 5-strain cocktail of L. monocytogenes to give ~10⁸ CFU/frankfurter, vacuum-packaged, and irradiated at 2.0 kGy. Samples were stored at 4°C for 90 days and analyzed for L. monocytogenes survivors at set intervals. Each frankfurter was washed in 20 ml of 0.1% peptone water and 0.1-ml aliquots were plated on Modified Oxford medium (MOX) agar. Survivors were determined by counting bacterial colonies on agar plates after incubation (35°C) for 48 hours. Based on initial numbers of L. monocytogenes recovered on control, numbers of the pathogen on samples (without SL) immersed in LQS, SLS, and LQS+SLS, were reduced by 0.66, 1.77, and 1.27 log, respectively. Log reductions on samples with SL were 0.77 (LS), 2.84 (SLS), and 2.13 (LS+SLS). Irradiation (2.0 kGy) reduced initial numbers of the pathogen on controls by 3.52 log and produced further reductions of 4.93,
2.60 and 4.26 log respectively, on LQS-, SLS-, and LQS+SLS-treated samples with no added SL. For samples with SL, irradiation reduced numbers by 4.15, 4.54, 3.43, and 3.65 for control, LQS-, SLS-, and LQS+SLS-treated samples, respectively. Growth of survivors was completely suppressed in all samples formulated with SL. Irradiation delayed growth of *L. monocytogenes* for 42 days in control and SLS-treated samples, and for 90 days in samples treated with LQS or LQS + SLS. Irradiation (2 kGy) of vacuum packaged frankfurters formulated with SL and surface-treated with LS or SLS is a good approach for controlling *L. monocytogenes* in these popular ready-to-eat meat products.

**INTRODUCTION**

*Listeria monocytogenes* is a foodborne pathogen that is ubiquitous in the natural environment and commonly found in the intestinal tract of infected animals, and in food processing plants (Ryser and Marth, 1999; Gillespie et al., 2000). This pathogen has a relatively high incidence on fresh meat and poultry (Farber and Peterkin, 1999). Because of its ubiquity in the environment, it is difficult to prevent its transmission from raw animal products to meat processing environments (Farber and Peterkin, 1999; Samelis and Metaxopoulos, 1999) and from the processing environment to ready-to-eat (RTE) meats (Tompkin et al., 1999). Because of its psychrotrophic characteristic *L. monocytogenes* can grow to high numbers in RTE meats during storage at refrigerator temperatures. Growth of the pathogen in refrigerated RTE cured meats is also linked to its ability to grow in the presence of curing salts at temperatures as low as 2°C (Lou and Yousef, 1999).

Several well-publicized listeriosis outbreaks have occurred in the United States. For example, a multistate outbreak from 1998 to 1999 was linked to frankfurters and deli meats
and caused 101 cases and 21 deaths (CDC, 1999). In 2000, a multistate outbreak attributed to consumption of deli turkey meat caused 29 cases, 4 deaths, and 3 miscarriages or still births (CDC, 2000). More recently, an outbreak in the northeast United States was linked to sliceable turkey deli meat. For this outbreak 46 confirmed cases, 7 deaths and 3 still births were reported (CDC, 2002). Among those hospitalized for contracting listeriosis, the fatality rate is between 20 to 30% (Ryser and Marth 1999, FAO/WHO 2004).

Food-related listeriosis is commonly associated with RTE foods such as luncheon meats, hot dogs, smoked fish, and some types of soft cheeses. Therefore, based on the characteristics of *L. monocytogenes* and severity of listeriosis, the Food and Drug Administration (FDA) and the U.S. Department of Agriculture Food Safety Inspection Service have specified a “zero” tolerance for this pathogen in RTE foods (Ryser and Marth, 1999; FAO/WHO, 2004). This zero tolerance policy has focused much attention on ways to control *L. monocytogenes* in RTE meat products which are commonly eaten without further heating by consumers. The microbial safety of RTE meats can be improved by incorporation of foodgrade additives that provide antimicrobial hurdles and prevent growth of *L. monocytogenes*. In this regard, antimicrobial food additives such as lactates, sorbate, and diacetate have be used in meat formulations (Bedie et al., 2001; Blom et al., 1997; Miller and Acuff, 1994; Mbandi and Shelef, 2002; Seman et al., 2002; Stekelenberg, 2003; Zhu et al., 2005) or applied to RTE meat surfaces (Islam et al., 2002a; Islam et al., 2002b; Samelis et al., 2002; Nunez De Gonzalez et al., 2004; Lu et al., 2005) to delay or prevent growth of *L. monocytogenes*.

Apart from organic acid salts, other antimicrobial food additives or processing aids seem to have good potential for controlling *L. monocytogenes* in RTE meat products. Liquid
smoke has been shown to be bactericidal to \textit{L. monocytogenes} (Messina et al., 1988; Faith et al., 1992; Poisky et al., 1997; Thurette et al., 1998; and Vitt et al, 2001). The bactericidal action is believed to involve components such as aldehydes, acetic acid, creosote, and phenolic compounds (Radecki et al, 1975). Sodium lauryl sulfate (SLS) is GRAS surfactant that is approved for food use. Among its many food applications are its uses as whipping agent in gelatin for marshmallows, an emulsifier in liquid and frozen egg whites, and a partitioning agent for animal fats and crude vegetable oils. When used in combination with citric acid and a chelating agent, SLS has been shown to reduce numbers of \textit{L. monocytogenes} on the surface of Formica counter by more than 5 log (Restaino et al., 1994). It is believed that the antibacterial action of SLS involves denaturing cell proteins, inactivating enzymes, and altering cell membrane permeability (Dychdala, 1983). Interestingly, anionic surfactants such as SLS are more effective against gram-positive than gram-negative bacteria (Cowles, 1938; and Baker et al, 1941). To our knowledge there are no published reports on the application of SLS in RTE meat products to control \textit{L. monocytogenes}.

Another strategy for controlling \textit{L. monocytogenes} in RTE meats is the application ionizing radiation (Institute of Food Science and Technology (IFST), 1999; Mendonca, 2002; Monk et al., 1995). Based on conclusions of a study group from the World Health Organization (WHO), food irradiated to a dose “appropriate to achieve the intended technological objective” is safe to eat and nutritionally adequate (WHO, 1994). Irradiation treatments can destroy foodborne pathogens, including \textit{L. monocytogenes}, and retard food spoilage (Olson, 1998). Although relatively high doses of irradiation can completely eliminate pathogens from RTE meat products, they are likely to negatively affect the
organoleptic qualities of these products (Sommers and Thayer, 2000). Therefore, the combined use of antimicrobial food additives and irradiation to provide a hurdle technology approach will permit the use of lower irradiation doses while providing an effective way to control *L. monocytogenes* in RTE meats.

To date, only few published studies describe the antibacterial efficacy of low to medium dose irradiation against *L. monocytogenes* in RTE meat products (Fu et al., 1995; Sommers and Fan, 2003; Chen et al., 2004; Foong et al, 2004; Zhu et al., 2005). Accordingly, the objective of this study was to evaluate the combined efficacy of electron-beam irradiation, liquid smoke, and SLS for controlling *L. monocytogenes* in frankfurters formulated with or without sodium lactate.

**MATERIALS AND METHODS**

**Preparation of frankfurters.** Frankfurters were prepared at the Iowa State University Meat Laboratory using meat trimmings made up of boneless beef (80:20 lean: fat ratio) and pork fat (20:80 lean: fat ratio). Both meat types were ground separately in a 0.79-cm grinder plate. The mixture of the beef trim, salt, sodium erythorbate, sodium nitrite, seasoning, and half the total water (added as ice) were chopped for 3 min in a vacuum chopper (Kutter Supplies, Inc., Randolph, MA). The basic formulation was used for preparing control frankfurters whereas separate batches of this formulation were mixed with sodium lactate (SL) to prepare meat batter with a final concentration of 2% (w/w) of the added SL. The pork trim, pork fat and ice were added when the temperature of the mixture reached 4.4°C (40°F). This mixture was chopped for 3 min until the temperature of the meat batter reached about 12°C (53.6°F). The meat batter was packed into 22-mm diameter peelable cellulose casings (Devro
TeepackWiener-Pak, Coastal Corrugated Inc., North Charleston, SC). The encased product was linked at 14 cm length x 2.2 cm diameter using a Poly-clip System (Kramer & Grebe GMBH & Co., Frankfurt, Germany).

The linked product was hung on racks and cooked in a humidity controlled smoke house (Alkar, DEC Intl. Inc., Lodi, WI) using a cooking-smoking cycle for 90 min. The final internal temperature of the cooked product was 71.1°C. The frankfurters were sprayed with cool water and chilled at 4°C for about 18-19 h. The next day, frankfurters were peeled with a peeler (Townsend Engineering Co., Des Moines, IA) then vacuum-packed in a Multivac 46800 (Multivac Sepp Haggenmuller, Gmbt & Co., Wolfertschwenden, Germany) and stored at -20°C in a walk-in freezer until used in experiments.

**Bacterial strains and preparation of inoculum.** A five strain mixture of *Listeria monocytogenes* was used to inoculate frankfurters used for the study. The strains used were Scott A NADC 2045, 101 M, H7776, 108 M, and F 6854. The Scott A NADC 2045 strain was obtained from the National Animal Disease Center (NADC) in Ames, IA. 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.

From each *L. monocytogenes* working culture, 0.1 ml was aseptically transferred to 10 ml of Tryptic Soy Broth supplemented with 0.6% yeast extract (TSBYE) (Difco) and incubated at 35°C for 18 h. A second transfer involved transferring 1 ml from each individual tube to separate flasks each containing 100 ml of TSBYE (Difco). The cultures were incubated at 35°C for 18 h. A 5-strain mixture of *L. monocytogenes* was prepared by
combining 6 ml of each culture to obtain a 30 ml mixture in a sterile 50-ml centrifuge tube. The cells were harvested by centrifugation (10,000 x g for 10 min at 4°C) in a Sorvall Super T21 centrifuge (DuPont Instruments, Wilmington, DE). The supernatant was discarded and the pelleted cells were resuspended in 0.1% peptone water (Difco) and washed once by centrifugation (10,000 x g, 10 min, 4°C). Pellets of washed cells were resuspended in 30 ml of fresh 0.1% peptone water to give a final cell concentration of approximately 10^9 colony forming units (CFU) per ml. These washed cells were used to inoculate the frankfurters.

**Preparation and application of surface treatments.** A 30% (v/v) solution liquid smoked of CharSol 24-P (Red Arrow Products Company LLC, Manitowoc, WI), a 1% (w/v) solution sodium lauryl sulfate (SLS) solution (Fisher Scientific International, Fair Lawn, NJ), and a combination of the two (LQS+SLS) were prepared for dipping. Frankfurters dipped in distilled water served as control. Frankfurters were dipped for two minutes in the assigned solutions, drained for 30 s, and aseptically placed in vacuum-package bags (Cyrovac B-2540, Cryovac Sealed Air Corp., Duncan, SC).

**Inoculation of samples.** Once frankfurters were placed in vacuum-packaging bags, each link was inoculated with 0.1 ml of washed cells to give a final concentration of 10^8 cells/link. The packaged frankfurters were manually massaged for 10 s to evenly spread the inoculum over their surfaces. Packages containing inoculated and non-inoculated frankfurters were packaged under vacuum in a Multivac A 300/51 vacuum packaging machine (Multivac Sepp Haggenmüller, GmbH & Co., Wolfertschwenden, Germany). All vacuum-packaged samples were held overnight (18-20 h) in a walk-in refrigerator (2-4°C) before subjected to irradiation.
**Electron-beam irradiation and dosimetry.** The vacuum packaged frankfurters were transported in a cooler containing crushed ice to the irradiation facility at Iowa State University Meat Laboratory one day after preparation. The inoculated and non-inoculated samples (4°C) were irradiated at 0 and 2 kGy. The samples were irradiated with a MeV CIRCE III Linear Electron Accelerator (MeV Industries S. A., Jouyen-Josas, France) operating at energy level of 10 MeV and an average dose rate of 62.2 kGy/min. The absorbed doses were measured by use of 5 mm (diameter) by 5 mm (length) alanine pellets (Bruker Analytische Messtechnik, Rheunstetten, Germany) that were positioned on the top and bottom sides of selected packages of frankfurters. Immediately after the irradiation process, the absorbed doses were measured by placing the alanine pellets in a Bruker EMS 104 EPR Analyzer (Bruker Analytische Messtechnik, Rheunstetten, Germany) which uses electronic paramagnetic resonance to achieve this measurement.

**Microbial analysis.** The samples were stored at 4°C and were analyzed for *L. monocytogenes* survivors on days 0, 14, 28, 42, 60 and 90. Randomly pulled samples were used for microbial analysis. Vacuum-packaged bags containing inoculated franks were aseptically opened and 20 ml of sterile peptone water was added to each package (Gabis et al., 1976). The packages were massaged vigorously by hand for 30 s and 1-ml aliquots of the wash solution were serially diluted in 0.1% peptone water. Aliquots (0.1 ml) of appropriate dilutions were surface-plated on Modified Oxford Medium (MOX) (Difco) agar plates. All inoculated agar plates were then incubated aerobically at 35°C for 48 h. The plates were removed from incubation after 48 h and colonies were enumerated using a Leica 3325
Quebec Darkfield Colony Counter (Leica, Buffalo, NY). Plates having 25 to 250 typical \( L.\) \textit{monocytogenes} colonies were counted, and counts were expressed as log CFU/link.

**Statistical analysis.** Three independent replicate experiments were conducted and two frankfurter samples per treatment were analyzed for each replication of the experiment. Analysis of variance (ANOVA) was performed with the General Linear Models procedure of the Statistical Analysis System software program (SAS Institute Inc., Cary, NC). Differences were considered statistically significant at \( P<0.05\) unless otherwise stated. Differences among variables were tested for significance using Tukey’s honestly significant different multiple comparison test. Mean values and standard error of the means (SEM) were reported.

**RESULTS**

Populations of \( L.\) \textit{monocytogenes} recovered from frankfurters (with no SL) that were immersed in water (control), LQS, SLS, or LQS + SLS are shown in Figure 3.1. Based on initial numbers of \( L.\) \textit{monocytogenes} on control frankfurters, numbers of the pathogen in samples (no SL) surface-treated with LQS, SLS, and LS + SLS, were reduced by 0.66, 1.77, and 1.27 log, respectively. \( L.\) \textit{monocytogenes} populations on samples formulated with SL and surface-treated with LQS, SLS, and LQS + SLS, were reduced by 0.77, 2.84, and 2.13 log, respectively. Relative to control, LQS treatment of frankfurters (formulated with or without SL) did not significantly reduce numbers of \( L.\) \textit{monocytogenes} (\( P > 0.05\)). Significant reductions were achieved only in frankfurters immersed in SLS or in LQS + SLS (\( P < 0.05\)). Frankfurters formulated with SL and immersed in SLS or LQS + SLS had
significantly lower numbers of survivors than samples formulated without SL and immersed in those treatment solutions (P < 0.05).

Figure 3.2 shows the effect of surface treatments on growth of *L. monocytogenes* in frankfurters formulated without SL and stored at 4 °C for 90 days. *L. monocytogenes* grew in water-dipped (control) frankfurters and in those dipped in SLS or LQS + SLS. In contrast, growth of the pathogen was inhibited for 90 days in LQS-treated frankfurters. Numbers of the pathogen increased relatively fast and reached 9.52 and 9.02 log CFU/frankfurter in control and SLS-treated samples, respectively, at 60 days. Growth of *L. monocytogenes* in frankfurters treated with SLS or LQS + SLS was delayed for 14 and 28 days, respectively. Populations of *L. monocytogenes* in frankfurters treated with LQS + SLS reached a maximum (7.82 log CFU) by day 42 and remained constant through 90 days. For frankfurters formulated with SL (Figure 3.3) no growth of the pathogen occurred for 90 days irrespective of the surface treatment.

Figure 3.4 shows the combined effects of surface treatments and irradiation (2 kGy) on growth of *L. monocytogenes* in frankfurters formulated without SL and stored at 4 °C for 90 days. Irradiation reduced numbers of *L. monocytogenes* on frankfurters surface-treated with water, LQS, SLS, and LQS + SLS, by 3.52, 4.93, 2.60, and 4.26 log, respectively. Growth of survivors in control and SLS-treated frankfurters was delayed for 42 days after which numbers of the pathogen increased to 8.74 log CFU (control) and 6.55 log CFU (SLS) at 90 days. In combination, surface treatment and irradiation resulted in total log reductions of 5.59, 4.37, and 5.53 in LQS-, SLS-, and LQS + SLS-treated frankfurters, respectively. No growth of survivors occurred in frankfurters that were treated with LQS or LQS + SLS.
The combined effects of surface treatments and irradiation (2 kGy) on growth of *L. monocytogenes* in frankfurters formulated with SL and stored at 4 °C for 90 days are shown in Figure 3.5. Irradiation alone reduced numbers of *L. monocytogenes* on frankfurters surface-treated with water, LQS, SLS, and LQS + SLS, by 4.15, 4.54, 3.43, and 3.65 log, respectively. Total log reductions in initial numbers of the pathogen due to surface treatment and irradiation were 5.31, 6.27, and 5.78 in LQS-, SLS-, and LQS + SLS-treated frankfurters, respectively. Survivors failed to grow in any of the irradiated frankfurters irrespective of the surface-treatment applied.

**DISCUSSION**

In the present study, significant reductions of *L. monocytogenes* on the surface of frankfurters were achieved by a 2.0 min immersion of the product in SLS or LQS + SLS. Sodium lauryl sulfate (SLS) is an anionic surfactant that is believed to exert its antibacterial action by denaturing cell proteins, and altering cell membrane permeability (Dychdala, 1983). The effectiveness of SLS against *L. monocytogenes* on frankfurters may also be attributed to its surfactant property. Because the surface of a frankfurter is hydrophobic and water is a poor wetting agent for hydrophobic surfaces it is difficult for hydrophilic antimicrobial solutions (without a surfactant) to inactivate microorganisms attached to fatty tissue or in fat smears. Also the strong attachment of *L. monocytogenes* to hydrophobic surfaces further exacerbates the problem of inactivating this organism on the surface of RTE meats. Foong et al (2004) demonstrated that 84 to 87% of *L. monocytogenes* cells attached strongly to RTE meats within 5 minutes. Also, *L. monocytogenes* attaches far stronger to fatty tissue than to lean meat tissue (Chung et al., 1989). Thus the surfactant characteristic of
SLS may permit better interaction of the antimicrobial with hydrophobic surfaces by emulsification of fat and increased wetting of those surfaces. This in turn may result in greater exposure of pathogens in fat tissue to the antimicrobial solution. Tamblyn and Conner (1997) reported that 0.5% lactic acid plus SLS (125 ppm) reduced numbers of firmly attached *Salmonella* on broiler skin by 1.3 logs. In general, anionic surfactants such as SLS are have been proven to be more effective against gram-positive than gram-negative bacteria (Cowles, 1938; and Baker et al, 1941).

Surface treatment of frankfurters with LQS alone did not yield significant reductions of *L. monocytogenes*. Potent antimicrobial activity of LQS has been demonstrated against *L. monocytogenes* in wiener exudate or laboratory broth medium (Faith et al., 1992), on cold smoked salmon (Vitt et al., 2000) and on frankfurters (Messina et al., 1998); however, under the conditions of the present study, LQS was not effective in reducing initial populations of *L. monocytogenes* on frankfurters (Figure 3.1). Differences in factors such as type of LQS, concentration, bacterial strains, dipping time and temperature used in the present and other studies most likely contributed to variations in results.

Although SLS produced a greater initial reduction of *L. monocytogenes* than LQS, SLS was ineffective in arresting growth of the pathogen during refrigerated storage of frankfurters formulated without SL. In contrast, LQS consistently prevented growth of *L. monocytogenes* during the entire storage period (90 days). Based on these results LQS (CharSol) has good potential for use as a growth inhibitor of *L. monocytogenes* in refrigerated frankfurters that might not contain lactates or lactate/diacetate combinations as bacteriostatic agents. Also, depending on the concentration and type of liquid smoke used as a dipping solution a bactericidal effect can be achieved. For example, Messina et al (1988)
reported that CharSol-10 was highly effective in controlling *L. monocytogenes* in vacuum packaged beef frankfurters; 99.9% of the initial population of the pathogen was destroyed 72 hours after dipping the product in full-strength CharSol-10.

The prevention of growth of *L. monocytogenes* for 90 days in refrigerated (4 °C) frankfurters that were formulated with SL was not surprising. Several published studies have reported the inhibitory action of sodium or potassium lactate against *L. monocytogenes* when those antimicrobials were used alone or in combination with other food additives in various product formulations (Bedie et al., 2001; Blom et al., 1997; Miller and Acuff, 1994; Mbandi and Shelef, 2002; Seman et al., 2002; Stekelenberg, 2003; Zhu et al., 2005). The primary mechanism is action of SL against bacteria including *L. monocytogenes* involves diffusion of the proton motive force by undissociated lactic acid formed from the organic acid salt (Eklund, 1985). Diffusion of the proton motive force causes inhibition of ATP synthesis and subsequent cessation of growth. While most of the SL remains in the anionic form, some amount of undissociated lactic acid can be formed considering the slightly acidic pH (6.0 to 6.2) of frankfurters used in the present study. Although the bacteriostatic property of SL is important for preventing growth of *L. monocytogenes* there is still an urgent need for an intervention step to destroy initial populations of this pathogen in RTE meats. In this regard, SL would continue to provide an extra layer of food safety by preventing growth of survivors.

In the present study the use of electron beam irradiation (2.0 kGy) proved effective in substantially reducing populations of *L. monocytogenes* on frankfurters. However, without SL in the product formulation or LQS on the product surface, *L. monocytogenes* can regain the ability to grow after a prolonged lag period (Figure 3.4). Therefore, the application of
ionizing radiation alone might not be a good strategy for controlling *L. monocytogenes* in RTE meats unless doses used are adequate for complete elimination of the pathogen without altering desirable sensory properties of the product. In the present study, surface-treatment of frankfurters with LQS followed by irradiation (2.0 kGy) resulted in greater than 4.0 log reduction of *L. monocytogenes*. Considering the fact that numbers of *L. monocytogenes* normally found in RTE meats are relatively low, ranging from < 10 to about 1,000 CFU/g (Buchanan et al., 1989; Johnson et al., 1990), a 4.0 log destruction of *L. monocytogenes* is adequate to eliminate this pathogen in frankfurters. Should there be any survivors, the presence of SL and low temperature (~4 °C) could serve as an additional barrier to their survival and growth (Figure 3.5).

There is a rapidly growing amount of published research on the addition of lactates, acetates and other antimicrobial food additives to the formulation of RTE meats. However, the effectiveness of these antimicrobials applied as spraying or dipping solutions for RTE meat products has yet to be fully exploited by the U.S. meat industry. Various European countries have long approved the use of sorbate and benzoate as dipping solutions for controlling growth of fungi on the surface of dry sausages (Sofos, 1989). To our knowledge there are no published studies that have evaluated a combination of LQS or SLS (as a surface treatment) and irradiation, as a post-processing intervention steps to control *L. monocytogenes* on cured meat products such as frankfurters. Post-processing application of antimicrobial interventions may be a more effective control strategy because the antimicrobial agent is applied directly on the surface of the meat product where *L. monocytogenes* cells are located.
This study has combined the use of chemical antimicrobials (applied both to the product surface and in the formulation) with irradiation to provide a multiple-hurdle preservation approach for control of *L. monocytogenes* in frankfurters. Further research is needed to optimize concentrations of chemical antimicrobial used as surface treatments and irradiation dose to destroy *L. monocytogenes* in RTE meats and maintain quality attributes of these popular meat products.

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**REFERENCES**


Figure 3.1. Populations of *Listeria monocytogenes* on frankfurters that were immersed (2.0 min) in treatment solutions (23±1°C), drained (30 seconds), then inoculated with $10^8$ CFU of the pathogen per frankfurters. Survivors were enumerated 24 hr after inoculation. SL = (sodium lactate); LQS = (liquid smoke); SLS = (sodium lauryl sulfate)
Figure 3.2. Populations of *Listeria monocytogenes* in non-irradiated (0 kGy) frankfurters formulated without sodium lactate (SL) stored at 4°C. LQS = (liquid smoke); SLS = (sodium lauryl sulfate)
Figure 3.3. Populations of *Listeria monocytogenes* in non-irradiated (0 kGy) frankfurters formulated with sodium lactate (SL) stored at 4°C.
LQS = (liquid smoke); SLS = (sodium lauryl sulfate)
Figure 3.4. Populations of *Listeria monocytogenes* in irradiated (2 kGy) frankfurters formulated without sodium lactate (SL) stored at 4°C. LQS = (liquid smoke); SLS = (sodium lauryl sulfate)
Figure 3.5. Populations of *Listeria monocytogenes* in irradiated (2 kGy) frankfurters formulated with sodium lactate (SL) stored at 4°C.
LQS = (liquid smoke); SLS = (sodium lauryl sulfate)
CHAPTER 4. GENERAL CONCLUSIONS

The use of 2 or 3% sodium lactate in the frankfurter formulation as included in this study prevents the growth of *L. monocytogenes* in vacuum packaged frankfurters under refrigeration (4 °C) or temperature abuse conditions (10 °C).

The use of either liquid smoke or sodium lauryl sulfate as surface treatments yielded reductions of >2.0 log CFU/frankfurter when applied to franks formulated with sodium lactate. Both products acted as bactericidal agents but only liquid smoke exerted a greater bacteriostatic effect against *L. monocytogenes*.

Electron-beam irradiation (2.0 kGy) is effective in reducing initial populations of *L. monocytogenes* and extending the lag phase of growth of the pathogen at 4 °C; however, the use of irradiation alone was not enough to prevent growth of the pathogen.

A combination of 2% (w/w) sodium lactate (in frankfurter formulation), 30% (v/v) liquid smoke or 1% (w/v) sodium lauryl sulfate (as a surface treatment), electron-beam irradiation (2 kGy), and cold temperature (4°C) is a highly effective strategy for controlling *L. monocytogenes* in frankfurters.

The combination of all of these interventions would insure that the threat of *L. monocytogenes* will be decreased substantially as opposed to using each intervention individually.
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