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Use of pediocin with other barriers for control of Listeria monocytogenes on ready-to-eat (RTE) processed meats

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Use of pediocin with other barriers for control of *Listeria monocytogenes* on ready-to-eat (RTE) processed meats

by

Chih-Ming Chen

A dissertation submitted to the graduate faculty
In partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Meat Science
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Iowa State University
Ames, Iowa
2003

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This is to certify that the doctoral dissertation of

Chih-Ming Chen

has met the dissertation requirement of Iowa State University

Signature was redacted for privacy.

Major Professor

Signature was redacted for privacy.

For the Major Program
DEDICATION

To my parents, parents-in law, wife, son, and younger brother.
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Frankfurters, in 1-link, 5-link and 10-link packages, were surface-inoculated with a 5-strain mixture of *Listeria monocytogenes* (3.40 or 5.20 log CFU/g) after treatment with 3,000 or 6,000 AU pediocin (in ALTA™ 2341) per link (Pdn-3000 or Pdn-6000). The frankfurters were vacuum-packaged, after which the packages were heated in hot water at 71°C, 81°C or 96°C for 30, 60 or 120 s or irradiated at 1.2 or 2.3 (single-link or 5-link packages), and 1.4 or 3.5 kGy (10-link packages). *L. monocytogenes* was enumerated following the treatments. Selected treatments were subsequently evaluated during storage at 4°C, 10°C and 25°C for up to 12 weeks.

*L. monocytogenes* on frankfurters was reduced by pediocin alone, by pediocin combined with post-packaging thermal pasteurization (PPTP) or by irradiation pasteurization (PPIP) for all package types. To achieve a 50% reduction or more of initial inoculation numbers, the following treatments were necessary: Pdn-6000 alone, Pdn-6000 plus PPTP (≥ 81°C, ≥ 60 s), Pdn-3000 and PPIP (≥ 1.2 kGy), or PPIP (≥ 2.3 kGy) alone. Frankfurters treated with Pdn-6000 alone inhibited the growth of *L. monocytogenes* at 4°C for 12 weeks. Significant antilisterial, and even listericidal, effects for the combinations of Pdn-6000 and PPTP (96°C, ≥ 60 s) or PPIP (≥ 2.3 kGy) were observed. PPTP and PPIP treatments significantly (P < 0.05) enhanced the antilisterial effects of pediocin. There was a significant (P < 0.05) synergistic effect between pediocin and irradiation that was not observed for pediocin and thermal treatments.

Use of pediocin and other barriers (PPTP or PPIP) are effective means to control the growth of *L. monocytogenes* during storage at refrigeration temperatures. These treatments had greater antilisterial effects during storage at 4°C than 10°C and 25°C thus temperature remains a critical point for control of this pathogen. Smaller package types (< 5 links per packages) resulted in greater antilisterial effectiveness for PPTP treatments. While dipping or spraying are effective methods for surface treatment of frankfurters with antimicrobials, other methods such as coated casings or co-extrusion systems are much easier to use. Further work with these systems to increase the microbial impact of the antimicrobials is recommended.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Recently, there have been several well-known outbreaks of Listeria infections from ready-to-eat (RTE) processed meat products (CDC, 1999a; CDC, 1999b; Farber and Peterkin, 1999). Frankfurters contaminated with Listeria monocytogenes have been found in the retail markets (CDC, 1998) in several instances. In the Bil Mar Foods outbreak that was announced on December 22, 1998, the manufacturer voluntarily recalled hot dogs and other meat products that may have been contaminated with L. monocytogenes as early as August 1998 (CDC, 1999a). The Center for Disease Control and Prevention (CDC) has reported that serotype 4b and ½a were isolated from frankfurters as well as deli meats (100 cases, 21 deaths, 22 states) (CDC, 1999b), and deli turkey meat (29 cases, 4 deaths, 3 miscarriages/stillbirths, 10 states) (CDC, 2000). It is imperative that the industry develops effective controls for L. monocytogenes, especially for RTE processed meats.

L. monocytogenes is a non-spore-forming linear rod that is a gram-positive, facultative, anaerobic psychrotrophic foodborne pathogen. Post-processing contamination of RTE processed meat products, such as frankfurters, by L. monocytogenes, represents a serious health risk (CDC, 1999a; CDC, 1999b). L. monocytogenes has become a major concern for the meat processing industry (Samelis et al., 2002) due to the high mortality rate associated with outbreaks and the lack of knowledge of the infectious dose for susceptible or immunocomprised individuals. Refrigeration temperatures and vacuum-packaging, which are two of the most common preservation techniques in the meat industry, do not inhibit the growth of L. monocytogenes (Glass and Doyle, 1989). The ability to grow at refrigeration temperatures (Glass and Doyle, 1989), as low as -0.1°C to -0.4°C (Walker et al., 1990), allows L. monocytogenes to persist in refrigerated RTE foods following contamination from the environment of processing plants. Applying low-temperature storage alone cannot keep meat safe from L. monocytogenes (Palumbo, 1986).

L. monocytogenes is widespread (Fenlon et al., 1996; Samelis and Metaxopoulos, 1999) and difficult to avoid before and during packaging (Bedie et al., 2001) due to frequent contamination of raw materials and plant environments. Therefore, the peeling procedure for frankfurters after heat pasteurization has been proposed as a point where easy contamination may begin for this pathogen. It has been suggested that the peeling operations must be considered a critical control point (CCP) in the Hazard Analysis and Critical Control Point (HACCP) program to reduce contamination of frankfurters (Wenger et al., 1990). However, additional hurdles in the form of pre- or post-packaging technologies may be necessary to control the growth of this pathogen and to enhance the safety of these products during storage (Bedie et al., 2001).

Samelis et al. (2001) emphasized that L. monocytogenes can be resistant to many food preservation methods (Lou and Yousef, 1999), colonize in meat plants (Samelis and Metaxopoulos, 1999) and survive under unfavorable conditions (Harmayani et al., 1993; Samelis and Metaxopoulos, 1999). L. monocytogenes has often been isolated from floors, drains, cleaning aids, walls, ceilings and critical control point areas in food processing facilities (Samelis and Metaxopoulos, 1999; Tompkin et al., 1999; Norrung, 2000; Tompkin, 2002). Extensive
efforts to control *L. monocytogenes* can reduce the level of contamination, but eradication from the processing environment or from all finished products may not be possible (Bernard and Scott, 1999). In general, *Listeria* can be controlled, but not completely eliminated, in the processing environment and in a broad variety of processed meats, included RTE meats, poultry products and luncheon meats (Tompkin 1983; Karr et al., 1994; Tompkin, 2002). Thus, post-packaging hurdle technologies are needed to inactivate or inhibit growth of *L. monocytogenes* in meat products during storage (Bernard and Scott, 1999).

Problems of *L. monocytogenes* contamination typically develop from post-thermal process contamination because the pathogen is relatively heat-susceptible. Therefore, it is feasible to inactivate this pathogen with mild heat treatment such as that given to minimally processed foods without negatively impacting the product quality. Roering et al. (1998) suggested that thermal pasteurization, at temperatures of 77°C or greater for at least 60 s, was sufficient to eliminate appreciable numbers of *L. monocytogenes*, regardless of other factors. Muriana et al. (2002) suggested that heating time of 2 min at 90.6°C to 96.1°C provided 2-log reduction of *L. monocytogenes* in most RTE deli meats and 4-log reduction if heated for 10 min. Assuming no further handling or processing of the products after being sealed, the pasteurization temperatures and times reported should be sufficient to eliminate relatively low levels of the pathogen resulting from post-processing contamination. Post-packaging thermal pasteurization (PPTP) may also have additional benefits; for example, reducing levels of *Escherichia coli* O157: H7 or *Salmonella* spp., on and/or within other types of sausage or processed meat products (Roering et al., 1998).

Post-processing intervention strategies, such as addition of pediocin (Ming et al., 1997) or organic acids (Samelis et al., 2001), treatments with thermal pasteurization (Roering et al., 1998), or irradiation (Fu et al., 1995b), are examples of methods which could be used to inactivate *L. monocytogenes* on RTE meat products. Pediocin, a bacteriocin (a peptide that is produced by some microorganisms during growth), has been found to inhibit several gram-positive bacteria including *L. monocytogenes*. For example, pediocin AcH can reduce or inhibit the growth of *L. monocytogenes* (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995). Pediocin AcH has been studied in a variety of fresh meats (Nielsen et al., 1990; Ming et al., 1997; Murray and Richard, 1997) and processed meat products, such as frankfurters (Berry et al., 1991), wiener (Degnan et al., 1992), and wiener exudates (Yousef et al., 1991). ALTA™ 2341, a commercial shelf-life extender and commercially available multifunctional food ingredient, possesses similar intrinsic listericidal activity (Schlyter et al. 1993b; Rozum and Maurer, 1997) to pediocin AcH.

Samelis et al. (2001) suggested that PPTP may increase the antilisterial effects of antimicrobials, such as pediocin, in cooked, cured meats. A 1- to 2-log reduction in surface contaminants of commercially manufactured products may be adequate to completely inactivate or reduce the pathogen populations to undetectable levels because contamination is typically relatively low. Such low levels of heat-stressed cells may be unable to recover and may also become more susceptible to current antimicrobials (Samelis et al., 2002).
In addition to the potential benefit of providing increased antilisterial effects, combinations of antimicrobials or other treatments may permit the use of lower concentrations of each and lessen any negative effects on the sensory quality of cured meat products (Samelis et al., 2002). Post-processing application of antimicrobials directly on the product surface where \textit{L. monocytogenes} cells may be present is more advantageous than their addition in the formulation (Tompkin et al., 1999).

Adequate absorbed doses of irradiation effectively kill disease-causing pathogens, including \textit{L. monocytogenes}, and delay food spoilage (Olson, 1998). Fu et al. (1995b) suggested that irradiation may provide an opportunity to reduce use of chemical additives because it decreases the load of microorganisms and eliminates some food pathogens. The authors also indicated that irradiation was effective for reduction of \textit{L. monocytogenes} on inoculated hams. They found that medium-dose irradiation at 2.0 kGy resulted in a population reduction of this pathogen to undetectable levels (6 logs) following inoculation. A low dose of 0.9 kGy achieved a 3-log reduction. However, the low dose did not eliminate the pathogen because some cells were able to recover when temperature was elevated to 25°C. Food irradiation may be considered a major means to meet the safety demands of consumers, in present and future meat and poultry markets (Radomyski et al., 1993).

Past research has shown that pediocin, PPTP and irradiation individually result in significant benefits for controlling the post-processing contamination of frankfurters by \textit{L. monocytogenes}. Combined treatments, such as spraying or dipping of products in antimicrobial (pediocin) solutions before packaging (Samelis et al., 2001), PPTP and irradiation (post-packaging treatment) (Fu et al., 1995b; Roering et al., 1998) may enhance the effectiveness of individual treatments for control of post-processing contamination with \textit{L. monocytogenes}. Therefore, meat plants should not rely solely on one method without previous evaluation of its effectiveness, particularly with different products. Also the quality of meat products may be influenced by different formulations and treatments introduced for pathogen control.

Applying multiple hurdle preservation approaches by treatment combinations of pediocin in conjunction with PPTP or post-packaging irradiation is a potentially good strategy to effectively control post-processing contamination of \textit{L. monocytogenes} on RTE processed meat products. Therefore, one objective of this study was to assess the inhibitory effectiveness of pediocin and combinations of pediocin (in ALTATM 2341) with PPTP and post-packaging irradiation for control of \textit{L. monocytogenes} on frankfurters. A second objective was to assess inhibitory effectiveness of selected antimicrobial treatments at 4°C, 10°C and 25°C during storage for up to 12 weeks. A third objective was to evaluate the physical and chemical quality, including sensory quality, of samples subjected to selected antimicrobial treatments. A final objective was to evaluate the practical application of these antimicrobial treatments to meat products, by comparing different delivery methods, such as surface application, co-extrusion of casings or use of casings treated directly with pediocin and other preservatives.
Dissertation Organization

This dissertation contains a general introduction, a literature review, three chapters on experimental work, and general conclusions. A general introduction is included at the beginning of Chapter 1. Chapter 2 includes a literature review on *L. monocytogenes*, pediocin, post-packaging thermal pasteurization, and post-packaging irradiation pasteurization. Three individual manuscripts, which are to be published, are individually designed as Chapter 3, 4, and 5. General conclusions including recommendations for future research comprises Chapter 6; followed by a list of references cited and acknowledgements at the end of this dissertation. The dissertation uses the standard format as prescribed by the thesis office at Iowa State University.
CHAPTER 2. LITERATURE REVIEW

Listeria monocytogenes

Introduction

Listeria monocytogenes is a non-spore-forming linear rod and gram-positive facultative anaerobic, psychrotrophic, foodborne pathogen that can be a major concern in the safety of ready-to-eat (RTE) meat products (CDC, 1999a; CDC, 1999b). It is well known that L. monocytogenes can survive on the surface of vacuum-packed frankfurters (Buncic et al., 1991) and grow at refrigeration temperatures (Glass and Doyle, 1989; Johnson et al., 1990). Low-temperature storage alone cannot keep meat safe from L. monocytogenes contamination (Palumbo, 1986). Zaika et al. (1990) reported that cooking frankfurters by heating them for 70 min to an internal temperature of 71°C (160°F) should kill L. monocytogenes (10^3 CFU/g or less). Wang and Muriana (1994) demonstrated that Listeria resulting from post-processing RTE product contamination was found only in the product exudate and was not detected in the interior of the product.

Moreover, the pathogen has been reported to persist in meat-processing environments, even after rigorous cleaning and sanitization (Samelis and Metaxopoulos, 1999). Even in well-run processing plants, cooked products may become contaminated before packaging; several cases have been documented (Wenger et al., 1990). The presence of L. monocytogenes in processing environments renders post-processed foods at risk for contamination sometime before or during packaging (Taormina and Beuchat, 2002). RTE meat products contaminated with L. monocytogenes are mainly contaminated after cooking and before packaging. For example, exposure of heat-processed frankfurters to the environment during peeling has been proposed as a point where contamination by this pathogen easily occurs (Wenger et al., 1990). Post-processing contamination of RTE processed meat products, such as frankfurters, with L. monocytogenes is difficult to avoid before or during packaging; thus, additional intervention hurdles are needed to enhance the safety of these products and to control growth of this pathogen during product storage (Bedie et al., 2001).

L. monocytogenes has been isolated from retail frankfurter samples (Wang and Muriana, 1994) and other vacuum-packaged processed meats (Grau and Vanderlinde, 1992). Therefore, Wenger et al. (1990) suggested that peeling operations must be considered critical control points (CCPs) in the Hazard Analysis and Critical Control Points (HACCP) program of such processing plants. Zaika et al. (1990) suggested that frankfurters would be a good model of RTE meat products for the survival investigations of L. monocytogenes, because frankfurters comprise the largest volume of cured meat products. Frankfurters also contain pork, beef, poultry and sometimes organ meats, and are salted, cured, and heated (cooked). Thus, processing of frankfurters is a suitable model for the study of L. monocytogenes survival.

Foodborne listeriosis

Listeriosis is known for a high mortality rate observed in several different outbreaks. There is also a lack of knowledge of the infectious dose for susceptible or immunocompromised individuals, such as neonates, cancer patients, the elderly, pregnant women and those with AIDS or other illnesses that affect the immune
system. Therefore, contamination of RTE meat products with \textit{L. monocytogenes} is a special threat to public health (Johnson et al., 1990). Interestingly, the results from surveys have found that between 0.5% and 2.5% of the healthy adult population are asymptomatic carriers (Fuchs, 1992). Most people who come in contact with the organism are not at risk of infection.

\textbf{Outbreaks of \textit{L. monocytogenes}}

\textbf{Incidence in the U.S.A.} Recently, there have been several well-publicized outbreaks of \textit{Listeria} infections from RTE processed meat products (CDC, 1999a; CDC, 1999b; Farber and Peterkin, 1999). On December 22, 1998, Bil Mar Foods voluntarily recalled hot dogs and other meat products that may have been contaminated with \textit{L. monocytogenes} as early as August 1998 (CDC, 1998). The case of Bil Mar Foods, in 1998 to 1999, was one of the most publicized, and one which resulted in a very large product recall (CDC, 1999a). The Center for Disease Control and Prevention (CDC) has reported that \textit{L. monocytogenes} serotypes 4b and 4a have been isolated from frankfurters, deli meats (100 cases, 21 deaths, 22 states) (CDC, 1999b) and deli turkey meat (29 cases, 4 deaths, 3 miscarriages/stillbirths, 10 states) (CDC, 2000). It is imperative that the industry develop effective controls for \textit{L. monocytogenes} on RTE processed meats.

Wang and Muriana (1994) found that 8\% (7 of 93) of the packages from 19 different brands of retail frankfurters tested positive for \textit{L. monocytogenes}. The pathogen was isolated from a variety of frankfurter types including chicken, beef/pork/turkey, beef and chicken/pork types. The results from several studies also agreed that retail wiener or frankfurters may support growth of \textit{L. monocytogenes}. This situation presents a potential hazard in consuming uncooked frankfurters or for cross-contamination of other foods from handling frankfurter package exudate (Glass and Doyle, 1989; Buncic et al., 1991; Mckellar et al., 1994; Wang and Muriana, 1994).

\textbf{Global Incidence.} The widespread occurrence of \textit{Listeria} makes this organism a global problem (Farber and Peterkin, 1999). According to the results of numerous surveys, \textit{L. monocytogenes} has been found in a variety of meats, including RTE processed meat products around the world. The areas of \textit{Listeria} incidence includes North America (the United States and Canada), Europe (Denmark, France, Germany, Italy, the United Kingdom, etc.), Asia (Australia, China, Japan, Taiwan, etc.) and South Africa.

\textbf{Incidence of \textit{L. monocytogenes} in meat products}

Sporadic contamination by pathogens may occur in slaughter houses and processing plants (Fu et al., 1995a). Even where Good Manufacturing Practices (GMPs) and HACCP systems have been emphasized (Tompkin, 1983; Karr et al., 1994), if proper processing has not been applied, pathogens can contaminate meat products, survive, grow and cause listeriosis.

Results from the United States Department of Agriculture–Food Safety Inspection Service (USDA-FSIS) \textit{Listeria-Monitoring programs for meat products found incidences of \textit{L. monocytogenes} in many products (Farber and Peterkin, 1999). \textit{L. monocytogenes} was isolated from raw meats (beef, ground beef, pork, and poultry), cooked and RTE meat products (cured ham, cooked roast beef, luncheon meats, fresh sausages, cooked smoked sausages), beef jerky, dry sausages and semidry fermented sausages.}
Incidence of *L. monocytogenes* in meat-processing facilities

*L. monocytogenes* is very difficult to control due to its ubiquitous characteristic in the environment in and around food plants and in homes, and is a frequent contaminant of raw materials, including meat or poultry. Thus, there is a constant reintroduction of the pathogen into the plant environment. Samelis et al. (2001) emphasized that *L. monocytogenes* can be resistant to many food preservation methods (Lou and Yousef, 1999), colonizes in meat plants (Samelis and Metaxopoulos, 1999) and survives under unfavorable conditions (Wenger et al., 1990; Harmayani et al., 1993; Samelis and Metaxopoulos, 1999). It has been documented that *L. monocytogenes* was often isolated from floors, drains, cleaning aids, walls and ceilings and CCP areas in food-processing facilities (Samelis and Metaxopoulos, 1999; Tompkin et al., 1999; Norrung, 2000; Tompkin, 2002).

In addition, Lunden et al. (2002) reported that a dicing machine, used for dicing cooked meat products in three plants, was found to transfer *L. monocytogenes* from one plant to another. Lunden et al. (2002) suggested that the problem cannot be completely eliminated from processed meats such as luncheon meats because the organism is widespread in food-processing environments. *Listeria* can be controlled but not eliminated from the cooked-processing environment based on results from 10 to 12 plants producing a broad variety of RTE meat and poultry products (Tompkin, 2002). Contamination of processed RTE foods with *L. monocytogenes* as a result of exposure in processing environments is a serious concern.

Floor surfaces are particularly difficult to render *Listeria*-free, and this provides a ready reservoir of this pathogen (Gravani, 1999). Refrigerated food plants, in particular, provide conditions that allow for survival and growth of *L. monocytogenes*. The refrigerated, moist environment, combined with organic soil deposition on floors, allows *L. monocytogenes* to survive and grow. This pathogen is constantly reintroduced into the plant environment by these means of entry.

Gravani (1999) and Tompkin et al. (1999) concluded that the current existing technology cannot eliminate *L. monocytogenes* from the cooked product environment of processing plants. Complete elimination of the pathogen from the meat-processing environment may be nearly impossible. The author also suggested that every potential point of entry and every potential cross-contamination source has to be monitored and controlled to control this pathogen.

Factors affecting the growth and survival of *L. monocytogenes*

Several environmental and processing conditions, such as temperature (pasteurization temperatures and storage temperatures), pH, atmosphere, food additives (sodium chloride, nitrite, nisin), and irradiation can influence the survival and growth of *L. monocytogenes* (Doyle, 1988; Johnson et al., 1990).

The composition of meat products. Growth of *L. monocytogenes* may vary with the composition of meat products (Grau and Vanderlinde, 1992; Taormina and Beuchat, 2002) such as the content of fat and concentration of sodium. Glass and Doyle (1989) observed differences in the growth rate of *L. monocytogenes* on wiener from different manufacturers. They suggested that antilisterial activity might be a result of phenols present in liquid smoke preparations.
Curing ingredients. Growth of *L. monocytogenes* is influenced by the content of ingredients in a formula, such as the amounts of residual nitrite (Grau and Vanderlinde, 1992; Schlyter et al., 1993b), polyphosphates (Giese, 1994) and phenols associated with smoke (Glass and Doyle, 1989; Glass et al., 2002). It has been reported that sodium nitrite works best when combined with other preservation strategies, especially low temperatures, pH 6.0 or below, and/or high salt contents (Buchanan et al., 1989; McClure et al., 1991; Schlyter et al., 1993b). Farber (1989) studied the heat resistance of *L. monocytogenes* in minced and sausage meat inoculated with the pathogen at the inoculation level of 1-2 × 10⁷ CFU/g. He found that sausage meat with curing ingredients caused greater D-values.

Salt. Two strains of *L. monocytogenes* were found to grow in trypticase soy broth (TSB) containing 10% NaCl at 35°C or 12% NaCl at 10°C and 25°C (Sorrells and Enigl, 1990). The pathogen may grow to high numbers in the presence of moderate amounts of salt. Lou and Yousef (1999) indicated that survival of *L. monocytogenes* may occur at a wide range of salt concentrations and vary with the storage temperatures.

Nitrite. Fu et al. (1995b) indicated that a high salt concentration (2.5%) added to cured ham products with sodium nitrite (140 ppm) probably contributed to inhibition of *L. monocytogenes*, especially when low to medium doses (≤ 2.0 kGy) of irradiation were applied. The authors also suggested that nitrite could have significant bacteriostatic effects on *L. monocytogenes*. Sodium nitrite is very effective when combined with other preservation means such as low pH, vacuum-packaging, high salt concentrations and adequate lower refrigeration temperatures (Buchanan et al., 1989; Sorrells et al., 1989; McClure et al., 1991; Schlyter et al., 1993b; Buncic et al., 1995). However, Junntila et al. (1989) concluded from their results that levels of nitrite and salt commonly used in the meat industry recently (120 ppm NaNO₂ and 3.0% NaCl) were not sufficient to eliminate *L. monocytogenes* (10⁵ CFU/g) from highly contaminated products.

Phosphates. Giese (1994) indicated that polyphosphate inhibits the growth of gram-positive bacteria, including *L. monocytogenes*. Trisodium phosphate (TSP) effectively inactivated *L. monocytogenes* in chicken meat, during refrigerated storage for several days (Capita et al., 2001). These authors inoculated chicken with 10⁶ CFU/ml *L. monocytogenes* and dipped the meat in 8% to 12% TSP solutions, then stored samples at 2°C for 5 days. For all sampling times, the best results were attained with the highest TSP concentration (12%). Reductions of the pathogen counts during the first day of storage and after 5-day storage were 1.52 logs and 2.70 logs or 2.10 logs and 3.63 logs, respectively, for 8% or 12% TSP-treated samples. But, polyphosphate (0.5%) alone, not accompanied with lower temperatures, did not prevent growth of *L. monocytogenes* (Buchanan et al., 1989; McClure et al., 1991).

Erythorbate. The industrial practice of applying antioxidants, such as sodium erythorbate, on the surface of cured meat products did not comprise the antilisterial efficiency of irradiation as an antimicrobial process (Sommers et al., 2002). The surface antioxidant concentration on cured meats did not reach a level sufficient to protect *L. monocytogenes* against the lethal effects of ionizing irradiation.

Biofilms. *L. monocytogenes* can attach to various surfaces on food-processing facilities and form biofilms (Bower and Daeschel, 1999; Tompkin, 2002). When biofilms are formed, they are hard to remove by
normal cleaning (Lou and Yousef, 1999). Biofilms also protect microorganisms, including *L. monocytogenes*, from antimicrobial or sanitizing agents and often serve as a source of recontamination for processed foods. Biofilm formation on the food-processing surfaces is an adaptive response to protect colonies from cleaning and sanitation (Bower and Daeschel, 1999; Tompkin, 2002). Mafu et al. (1990) found that *L. monocytogenes* attached to stainless steel, glass, polypropylene and rubber, which are common materials in food-contact surfaces, after short contact times (20 to 60 min) at both ambient and refrigeration temperatures. Microorganisms embedded in biofilms are more resistant to heat, sanitizers and other antimicrobial agents than are freely suspended (planktonic) cells (Frank and Koffi, 1990).

Applying multiple hurdle preservation approaches and preventing bacterial adhesion are effective strategies to counter resistance development (Bower and Daeschel, 1999). If bacterial adhesion does occur, then a bacteria-filled biofilm may result. To prevent the development of resistant strains of bacteria forming on food-contact surfaces, it is essential to maintain cleanliness. In addition, proper equipment design is essential for avoiding cracks and dead areas where organic material could accumulate because crevices and other surface imperfections shield attached cells from the rigors of cleaning procedures. Application of multiple hurdle interventions can reduce the possibility of bacterial survival in the food-processing environment and prevent subsequent microbial growth and reproduction. To accomplish this, preservation methods are combined to create a series of “hurdles” throughout the process with each representing a barrier that must be overcome by the bacteria to survive and/or grow.

**Strains of *L. monocytogenes***. The surviving populations of *L. monocytogenes* in the study by Porto et al. (2002), who used a 5-strain mixture of *L. monocytogenes* for inoculation, were lower than those from the study by Samelis et al. (2002), who used a 10-strain mixture of *L. monocytogenes*. Different strains of *L. monocytogenes* cultures have resulted in different survival rates (Porto et al., 2002; Samelis et al., 2002).

**Sublethal stress resistance**

**Heat-shock response.** Thermal processing is the most widely used method to preserve food, destroy harmful microorganisms, and render meat products safe for human consumption (Lou and Yousef, 1999). It is well known that environmental stresses can induce stress-adaptive or stress-protective responses. Incubating a microorganism at a high but sublethal temperature will induce heat-shock adaptive response. Due to stress adaptation, which occurs in all bacteria, the resistance of *L. monocytogenes* to heat or other lethal factors can be markedly increased. Farber (1989) suggested that the “heat-shock” effect could be significant for bulk foods that are heated slowly or other foods that receive a marginal heat treatment. Bacteria respond to heat shocking by synthesizing new proteins, termed heat-shock proteins (HSPs). Induction of the heat-shock response or HSPs usually increases the thermotolerance of microorganisms. Heat-shock-induced thermotolerance is transient and non-heritable; therefore, it is an acquired or adaptive thermotolerance. Quintavalla and Campanini (1991) observed that thermotolerance of *L. monocytogenes* in a pork emulsion was two or three times more than it was in broth. Heat resistance of microorganisms also can be markedly increased by the addition of NaCl and sucrose in the heating menstruum. Curing ingredients increased heat resistance of the pathogen in beef samples
(Mackey et al., 1990) because these conditions provide sublethal stresses for surviving *L. monocytogenes*. The surviving cells are injured from some sublethal stresses, but not killed. The survivors then induce HSPs to increase thermotolerance of surviving *L. monocytogenes*.

**Cleaning sanitizers.** Bacterial cells may become more resistant to subsequent antimicrobial treatments by forming a biofilm on food-processing surfaces. Biofilms have been found to be an adaptive response to protect colonies from cleaning and sanitation (Bower and Daeschel, 1999). Taormina and Beuchat (2002) suggested that cells that escape subsequent exposure to sanitizers after being exposed to alkaline cleaners or detergents would not be inhibited on foods stored at refrigeration temperatures. Bower and Daeschel (1999) also indicated that applying multiple hurdle preservation approaches reduces resistance development of pathogens and is one of the major strategies to effectively control pathogens.

Unfortunately, sanitation strategies and hygienic practices applied in plants are often insufficient to prevent recontamination and growth of *L. monocytogenes* in processed meat products (Samelis and Metaxopoulos, 1999; Tompkin et al., 1999). Extensive efforts to control *L. monocytogenes* can reduce the level of contamination, but it has not been possible to eradicate it from the processing environment or from all finished products (Bernard and Scott, 1999).

**Storage temperatures**

Glass and Doyle (1989) found that *L. monocytogenes* grew at 4.4°C on ham, bologna, sliced chicken and turkey products, wieners and fresh bratwurst. They emphasized the importance of preventing post-processing contamination of RTE meat products with *L. monocytogenes*. The long-held premise that refrigerated storage at 4°C to 7°C will prevent the growth of foodborne pathogens clearly is not valid for this organism. Meat manufacturers can no longer rely completely on refrigerated storage at 4°C to 7°C to be assured of pathogen control (Palumbo et al., 1986). Thus, Glass and Doyle (1989) recommended novel, nontraditional measures, such as the use of antimicrobial agents, reduced temperature (< 2°C) storage, reformulation of products or post-processing pasteurization of products for the control of *L. monocytogenes* in meats.

Refrigeration temperatures and vacuum-packaging, two of most common techniques used in the meat industry, do not inhibit the growth of this pathogenic microorganism (Junttila et al., 1988; Glass and Doyle, 1989). Its ability to grow at refrigeration temperatures (Junttila et al., 1988; Glass and Doyle, 1989) as low as -0.1°C to -0.4°C (Walker et al., 1990) and the ubiquitous character of *L. monocytogenes* (Fenlon et al., 1996; Samelis and Metaxopoulos, 1999) allow this organism to survive these processes. These special characteristics make it easier for *L. monocytogenes* to contaminate RTE foods in the plant environment after cooking or thermal-processing steps. Thus, the pathogen can subsequently survive for long periods in different environments.

As *L. monocytogenes* is common in food-processing environments (Cox et al., 1989, as cited in Gravani, 1999), the microorganism may be adapted to grow at low temperatures before contamination of the food in storage. Consequently, the safety margins of food storage at low temperatures may be substantially
smaller than those indicated by studies that have not considered the potential for cells to adapt to growth at low temperatures. Several studies have pointed out that refrigerated temperatures (0°C to 10°C) do not prevent the growth of *L. monocytogenes*; but will still extend the time before growth occurs and will reduce the rate of growth. These effects become greater with lower storage temperatures (Walker et al., 1990).

A study by Buncic et al. (1991) indicated that *L. monocytogenes* can survive on the surface of vacuum-packaged frankfurters with little growth inhibition. They found that the pathogen increased from $5 \times 10^2$ to $2.1 \times 10^5$ CFU/g during storage for 20 days when surface-contaminated frankfurters were stored at 4°C in vacuum packages. Glass and Doyle (1989), who studied ham, bologna, sliced chicken and turkey products, wieners and fresh bratwurst reported that growth of *L. monocytogenes* at 4.4°C depended greatly on the type of product. Grau and Vanderlinde (1992) indicated that refrigeration temperatures (0°C to 5°C) enhanced the antilisterial effects of high residual nitrite and reduced the growth of *L. monocytogenes*. Growth of surviving cells of *L. monocytogenes* has been found to be more rapid on pediocin-treated samples stored at 10°C than that on those stored at 4°C (Motlagh et al., 1992; Porto et al., 2002).

Even though *L. monocytogenes* can grow very well at the lower refrigerated temperatures, such as 4°C, the pathogen may not survive or grow well if cells are seriously injured after treatments, such as addition of pediocin. Glass et al. (2002) reported that a lower temperature enhanced the antilisterial activity of organic acids on unsmoked bratwurst (P < 0.05). Therefore, refrigerated temperatures are still a critical factor to control the growth of *L. monocytogenes*.

### Seasonality

Seasonality has been shown to affect occurrences of *L. monocytogenes* (Tompkin, 2002). The author indicated that there was a higher prevalence rate of this pathogen in the processing environment during the summer months.

### Pediocin

#### Introduction

The antilisterial effectiveness of pediocin has been demonstrated in different kinds of meat products by several studies (Nielsen et al., 1990; Berry et al., 1991; Degnan et al., 1992). The results from several investigations showed that pediocin can reduce or inhibit the growth of *L. monocytogenes* (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995). Post-packaging intervention strategies, such as addition of pediocin (Pucci et al., 1988), have been shown to inactivate *L. monocytogenes* on RTE meat products.

#### General properties of pediocin

Pediocin is one of the bacteriocins produced by some lactic acid-producing bacteria during growth. Bacteriocins are peptides that demonstrate antimicrobial activity. Pediocin, for example, has been found to inhibit several gram-positive bacteria, including *L. monocytogenes* (Lou and Yousef, 1999). Although most bacteriocins have a narrow spectrum of inhibition and only inhibit closely related species, pediocins have a
relatively broad spectrum and can inhibit some less closely related organisms. Even though modes of action of bacteriocins vary, bacteriocins usually destabilize the cytoplasmic membrane of sensitive cells, increase membrane permeability and dissipate the proton motive force (PMF) by forming water-filled transmembrane pores or channels (Jack et al., 1995).

Pediocin AcH is a peptide of about 3 kDa, which is identical to pediocin PA-1 (Motlagh et al., 1992). Several studies have reported that pediocin can reduce or inhibit the growth of *L. monocytogenes* (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995). Pucci et al. (1988) added pediocin PA-1 powder, at a final concentration of either 200 or 500 AU/ml, to exponential-phase cultures of *L. monocytogenes* LM01 to examine the effect on growth over storage time. These authors observed that a decrease in turbidity over time was indicative of cellular lysis and inhibition by pediocin PA-1. This pediocin was effective from pH 5.5 to 7.0 and at both 4°C and 32°C. The data in this study indicated that *L. monocytogenes* is sensitive to the effects of pediocin PA-1. It was evident that inhibition occurred over a wide range of temperature and pH, and was particularly effective at a low initial inoculation of the pathogen, similar to what could occur in various food systems. Pediocin AcH has been reported to be resistant to heat for 15 min at 121°C (Bhunia et al., 1988).

**Advantages and disadvantages for food applications**

Bacteriocins produced from cultures of lactic acid-producing bacteria have been present in fermented food products for centuries (Nielsen et al., 1990). Thus, addition of pediocin to foods should pose no risk to consumers. For example, pediocin AcH and PA-1 are produced by *Pediococcus acidilactici* strain H (Bhunia et al., 1988) and *P. acidilactici* PAC 1.0 (Gonzalez and Kunka, 1987), respectively, isolated from fermented sausages. Pediocin can provide a relatively broad spectrum of inhibitory activity against gram-positive bacteria (Gonzalez and Kunka, 1987; Jack et al., 1995). Pucci (1988) suggested that bacteriocins offer safe and natural compounds for controlling undesirable bacterial contamination, such as *L. monocytogenes*. There are some potential disadvantages to using pediocin in meats. These include inactivation of the pediocin by specific proteases of products, non-specific binding to food, limited diffusion in a solid matrix, inducible resistance to pediocin (Murray and Richard, 1997) and limited effects on gram-negative bacteria (Spelhaug and Harlander, 1989; Schved et al., 1994). Nisin has been approved as a food additive in many countries, including the United States, for years (FDA, 1998). However, pediocins have not yet been approved by the FDA for use in meat products because they are not generally considered safe (GRAS) ingredients. Thus, pediocin is not allowed for direct use in foods. Resistance to the antimicrobial action of nisin has been demonstrated (Mazzotta and Montville, 1997; Crandall and Montville, 1998). For example, a strain of nisin-resistant *L. monocytogenes* was found that had a lower ratio of C15 to C17 fatty acids than the normal strains, as well as an altered requirement for divalent cations. These genetic changes confer resistance by somehow disrupting the normal inhibitory mechanism of nisin. While, *L. monocytogenes* survivors were found to become resistant to nisin, survivors of pediocin AcH remained susceptible to this bacteriocin (Murray and Richard, 1997). However, Song and Richard (1997) found that nisin-resistant cells resulted in cross-resistance to other bacteriocins, including
pediocin. Cross-resistance to pediocin PA-1 has also been displayed by nisin-resistant strains of *L. monocytogenes* (Mazzotta et al., 1997). Nisin displays the fastest inhibitory activity among bacteriocins, including pediocin (Song and Richard, 1997), but the activity of nisin is more ATP-energy dependent than that of pediocin (Bruno and Montville, 1993). Thus, it is likely that *L. monocytogenes* is not killed by nisin, but is more likely to be destroyed by pediocin AcH. The findings from several studies using model food systems demonstrated that pediocin-like bacteriocins are effective for killing pathogenic microorganisms in meat products where nisin is ineffective (Nielsen et al., 1990; Jack et al., 1995).

**Types of pediocin for product applications**

**Inoculation with pediocin-producing starter cultures.** Some researchers have tried to directly inoculate products such as frankfurters with pediocin-producing starter cultures, such as *P. acidilactici*, to produce pediocin in-situ and inhibit *L. monocytogenes* (Berry et al., 1991; Degnan et al., 1992; Foegeding et al., 1992). Berry et al. (1991) reported that *P. acidilactici* JDI-23 at low or high level (10^3-10^4 CFU/g or 10^7 CFU/g) extended the lag-phase of growth of *L. monocytogenes* on frankfurters inoculated with of 10^4 CFU/g at 4°C. *P. acidilactici* (10^7 CFU/g) inhibited growth of *L. monocytogenes* for up to 60 days, although no population reduction of the pathogen occurred. But the other strain of *P. acidilactici* JBL 1095 (pediocin AcH producer) did not produce acid or pediocin during storage at 4°C (Degnan et al., 1992). *L. monocytogenes* was inhibited (ca. 2.7-log reduction) in packages inoculated with strain JBL 1095 during storage at 25°C for 8 days. It was observed that pediocin is produced and has an effective antilisterial activity during fermentation of dry sausage also (Foegeding et al., 1992).

These studies suggested that control of *L. monocytogenes* was improved by in-situ produced pediocin, but the selection of *P. acidilactici* strains needs to be considered. Pediocin production can contribute to the safety of meat products (Berry et al., 1991; Degnan et al., 1992; Foegeding et al., 1992).

**Encapsulation of pediocin.** Bacteriocin activity can be adversely affected by certain food components (Degnan and Luchansky, 1992). The data showed that pediocin AcH activity had significant loss within 1.5 min (only 24-60% recovery) following the addition of 30,000 AU of pediocin per ml of slurries (25% in dH_2O) of beef tallow and muscle. Degnan et al. (1993) employed encapsulation techniques to improve pediocin activity in food slurries. Pediocin, which was encapsulated in phosphatidyl-choline-based liposomes or combined with 0.12% Tween 80, increased pediocin activity to 29-62% from 12-54% of original activity.

**Pediocin in killed cells.** Pediocin bound to heat-killed cells of the producer organism still showed strong activity for antilisterial effects on irradiated raw chicken breast meat stored at 5°C (Goff et al., 1996). These authors reported that a pediocin level of 2,400 AU reduced the *L. monocytogenes* count by 2.2 logs for storage of 28 days with an initial inoculum of about 5 log CFU/g of *L. monocytogenes* Scott A. With a lower initial inoculation level of ca. 3 log CFU/g of this pathogen, addition of the same concentration of pediocin provided a 1-log reduction at day 0, and the counts become less than detectable from day 7 to 28.
**Purified pediocin.** Purified pediocin AcH has demonstrated inhibitory activity against a range of gram-positive bacteria, including *L. monocytogenes* (Bhunia et al., 1988). Dried pediocin PA-1 has been effectively applied to inhibit *L. monocytogenes* in a variety of foods (Pucci et al., 1988).

**Commercial products available – ALTA™ 2341.** ALTA™ 2341, which is a commercially available shelf-life extender (Rozum and Maurer, 1997) and multifunctional food ingredient, possesses similar intrinsic listericidal activity similar to pediocin (Schlyter et al., 1993a). Szabo and Cahill (1998) reported that a seven-strain cocktail mixture of *L. monocytogenes* was used to assess the antilisterial effectiveness of the ALTA™ 2341 in combination with various atmospheres: air, 100% N₂, 40% CO₂(g): 60% N₂(g), or 100% CO₂(g). The *L. monocytogenes* cocktail was grown in buffered tryptone soy broth (pH 6.0) and held at 4°C (21 days) or 12°C (7 days). Addition of 1% ALTA™ 2341 in this buffered broth to inoculated *L. monocytogenes* resulted in a population decrease to below the detection level within 24 h when combined with all atmospheres at different storage temperatures (4°C and 12°C).

**Mechanisms of pediocin inhibition of *L. monocytogenes***

Generally, the primary target of bacteriocins is the cytoplasmic membrane. To perturb bacterial membranes, the cysteine residues in the structure of the bacteriocin molecule (Miller et al., 1998; Montville and Chen, 1998) often display properties of an amphipathic α-helix (Nissen-Meyer et al., 1992), which makes the bacteriocin molecules initiate rearrangements in the membrane to make pores in the membranes. This process disturbs membrane transport, dissipates the PMF and thus leads to inhibition of energy production (Montville and Bruno, 1994).

**Membrane lysis.** Pediocin PA-1 ultimately caused concomitant cell death of *L. monocytogenes* Scott A (Tagg et al., 1976; Chen and Montville, 1995). Bhunia et al. (1991) reported that one manifestation of the lethal effect of pediocin AcH may be membrane damage that is severe enough to cause lysis of the cells. Similar results were reported for pediocin PA-1 effects on *L. monocytogenes* (Pucci et al., 1988), as discussed above. Study of the detailed molecular effects of pediocin PA-1 indicated that two disulfide bonds (cysteine residues), which are present in the pediocin molecule, are essential for its antimicrobial activity (Miller et al., 1998). This differs from nisin with respect to the time scale required for action and the size of pore formed in the cytoplasmic membrane during the action.

**Proton motive force (PMF).** Pediocin PA-1 and other bacteriocins produced from lactic acid bacteria share a common mechanism that dissipates PMF in *L. monocytogenes* (Christensen and Hutkins, 1992; Bruno and Montville, 1993). Chen and Montville (1995) demonstrated that pediocin PA-1, like nisin, induced irreversible K⁺ efflux, which is pediocin concentration- and action time-dependent. This causes cells of *L. monocytogenes* Scott A to lose intracellular K⁺. Because the fundamental role of PMF is in bacterial energy metabolism, PMF dissipation would ultimately cause the death of the cell. Low intracellular levels of ATP (adenosine triphosphate), the inability to carry out the active transport of nutrients and the inability to maintain sufficient concentrations of cofactors, such as K⁺ and Mg²⁺, are direct results of PMF collapse, and would all contribute to growth inhibition and death of the cells.
Cytoplasmic ATP (U.V.-absorbing materials) in cells. In addition to showing induced irreversible K⁺ efflux, Chen and Montville (1995) have also demonstrated that pediocin PA-1 induced inorganic phosphate (Pi) efflux, but not ATP, from *Listeria* cells. The Pi efflux was from the cytoplasmic ATP (U.V.-absorbing materials) of cells of *L. monocytogenes* Scott A (sensitive bacteria), and was pediocin concentration-dependent and action time-dependent. Moreover, pediocin PA-1 depleted the majority of total cellular ATP prior to significant phosphate efflux.

**Glucose uptake.** Christensen and Hutkins (1994) concluded that *L. monocytogenes* Scott A accumulates glucose by phosphotransferase and PMF-mediated systems, both of which are sensitive to pediocin JD. Thus, glucose uptake by *L. monocytogenes* Scott A was inhibited by pediocin.

Bhunia et al. (1991) tried to explain the possible mechanism of action for pediocin AcH in sensitive cells as follows: (1) initially pediocin AcH binds to the non-specific receptors, probably lipoteichoic acid; (2) when the non-specific sites are saturated, pediocin AcH molecules bind to specific receptor(s) and bring about changes in the membrane integrity; (3) as a result of functional disintegrity of the membrane, the cell loses potassium ions, U.V.-absorbing materials and other small molecules, and also loses the ability to multiply; (4) the cell membrane in some strains also loses structural integrity and lyases; (5) the specific receptors are either absent or not available for binding in the resistant gram-positive bacteria; and (6) both non-specific and specific receptors for pediocin AcH in the gram-negative bacteria are absent.

**Antilisterial effectiveness of pediocin in variety of meat products**

Pediocin has been studied for its antilisterial effects (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995) in a variety of fresh meats (Nielsen et al., 1990) and processed meat products (Berry et al., 1991; Yousef et al., 1991; Degnan et al., 1992). The bactericidal action of pediocin AcH against *Listeria* spp. was rapid and was not adversely affected by the meat used in a study by Motlagh et al. (1992). A study by Schlyter et al. (1993b) reported that the addition of pediocin (5,000 AU/ml) to turkey slurries provided an immediate 0.9 log CFU/ml decrease from initial inoculation of 4.5 log CFU/ml *L. monocytogenes*.

**Fresh meats.** A study by Nielsen et al. (1990) indicated that beef samples contaminated with *L. monocytogenes* and then treated with pediocin resulted in attachment of 1.0 to 2.5 logs fewer organisms than were attained with the control samples. The reduction of the number of attached *L. monocytogenes* was dependent on the concentrations of pediocin and the pathogen. The reduction of the pathogen was greater with increased concentrations of pediocin, similar to results reported by Motlagh et al. (1992). Inhibitory effectiveness of pediocin on *L. monocytogenes* with a large inoculum of around 7.0 log CFU/ml was, in decreasing order, 5,000 AU/ml (2.0 log CFU/g reduction), > 1,000 AU/ml (> 1.0 log CFU/g reduction) > 500 AU/ml (< 1.0 log CFU/g).

**Processed meat products.** In vacuum packages of surface-inoculated frankfurters, the MPN of *L. monocytogenes* increased from $5 \times 10^2$ to $2.1 \times 10^5$ per package during 20 days of storage at 4°C (Buncic et al.,
1991). This finding confirms previously reported data that vacuum-packaged meat products, in anaerobic conditions, represent a favorable environment for the pathogen (Buncic et al., 1991).

Applying pediocin to processed meat products was useful, especially for RTE meat products, including frankfurters (Berry et al., 1991), wiener (Degnan et al., 1992), and wiener exudates (Yousef et al., 1991). The population of \textit{L. monocytogenes} on vacuum-packaged beef wiener decreased by 2.7 logs in the presence of the pediocin-producing \textit{P. acidilactici} compared with populations on untreated samples, which increased by 3.2 log CFU/g (Degnan et al., 1992). Berry et al. (1991) found that the bacteriocin-producing strain \textit{P. acidilactici} JDI-23 provided effective protection for vacuum-packaged frankfurters from \textit{L. monocytogenes} during storage at 4°C.

Fermented sausages. Control of \textit{L. monocytogenes} by pediocin-producing starter cultures has been observed during the manufacture of dry or semidry sausage. Buncic et al. (1991) reported that the numbers of \textit{L. monocytogenes} on fermented sausage with cultures of 12 \textit{Lactobacillus plantarum} strains decreased from $8 \times 10^6$ to $1.1 \times 10^4$ during ripening and drying. Berry et al. (1990) reported a 2-log reduction in numbers of \textit{L. monocytogenes} using a bacteriocin-producing strain of \textit{Pediococcus} and < 1-log reduction with a nonbacteriocinogenic starter during fermentation of semidry sausage. Foegeding et al. (1992) compared the antilisterial activity of two starters, a pediocin-producer, \textit{P. acidilactici} PAC 1.0, and its isogenic pediocin-negative derivative, during dry sausage production. The investigators observed that pediocin produced \textit{in-situ} was partially responsible for listeriae inactivation during the sausage fermentation and drying.

Practical applications of pediocin

Spraying the surface of meat products. Glass et al. (2002) suggested that surface application of antimicrobials had less impact on the sensory and functional properties of meat products compared with inclusion of antimicrobials in the product formulation. Surface treatment of the cooked meat products with antimicrobial solutions can provide secondary barriers and delivers the antimicrobial agents at the point of contamination after chilling and before packaging. The safety of RTE meats can be enhanced when this barrier system is used in combination with diligent HACCP and environmental \textit{L. monocytogenes} control programs.

Except for the direct means of adding pediocin or other antimicrobials to the surface of processed meat products by spraying and dipping, the indirect approaches, in which the bacteriocin or antimicrobials are applied to food packaging materials (Ming et al., 1997), has potential for the industry. Wang and Muriana (1994) reported that pediocin, which has been proposed as a "biopreservative", serves as an additional barrier to control microorganisms in foods. Pediocin can be used as one of the barriers to \textit{L. monocytogenes} to enhance safety. Spraying pediocin on the surface of frankfurters to inhibit the growth of \textit{L. monocytogenes} before sealing packages, is simple and economical to perform.

Spraying interior of casing or inner side of packages. Recently, a study described application of antimicrobials, such as pediocin at 9.30 µg/cm², on the internal surface of cellulosic casings, to inactivate \textit{L. monocytogenes} ($10^5$ to $10^6$ CFU/ml) on meat products (Ming et al., 1997). These authors suggested that pediocin-coated casings could provide an effective antimicrobial barrier on food surfaces. The researchers in
This study also evaluated application of antimicrobials on the internal layers of packages (5 AU/cm²) to inactivate *L. monocytogenes* on meat products (Ming et al., 1997). The authors reported that the bags coated with pediocin powder completely inhibited growth of inoculated *L. monocytogenes* (1,000-5,000 CFU/item) on fresh beef and fresh turkey breasts but not on ham, during storage for 12 weeks at 4°C. Thus, they suggested that applying bacteriocins to food packaging films is an effective means to reduce *L. monocytogenes* contamination on meats and poultry products.

**Added into biodegradable packaging, edible films or casings.** Antimicrobials, such as nisin or organic acids, have been added to edible films (Ko et al., 2001), biodegradable packaging films (Padgett et al., 1998) and antimicrobial food packaging systems (Han, 2000). The results from the studies by Ko et al. (2001) reported that edible films with added nisin formed excellent films and displayed effective inhibition of bacterial growth of *L. monocytogenes* V7 serotype (1/2a). But, interactions between nisin and proteins, which are the major sources of the different films, including soy protein isolates, spray-dried wheat gluten, egg albumen and whey protein isolate, resulted in variation in the mechanical properties and water vapor permeabilities of the films tested. More research is needed to improve the mechanical properties of the films.

Adding pediocin to co-extruded collagen casings is a novel idea to achieve inhibition of *L. monocytogenes*. This approach would protect frankfurters from contamination by *L. monocytogenes* by forming a protective coating of pediocin on the surface of frankfurters.

Because outbreaks of *L. monocytogenes* happen sporadically and unpredictably, additional protective measures are necessary to help to control *L. monocytogenes* or at least to reduce the impact of *L. monocytogenes* hazard. Research using pediocins as intervention methods for surface-treated frankfurters has been limited. Post-processing application of antimicrobials applied directly on the product surface where contaminating *L. monocytogenes* cells are more likely to exist may be more advantageous than their addition in the formulation (Tompkin et al., 1999). In addition to the potential for providing increased antilisterial effects, combinations of antimicrobials or treatments may lessen any negative effects on the sensory quality of cured meat products (Samelis et al., 2002).

Nielsen et al. (1990) indicated that bacteriocins from lactic acid-producing bacterial starter cultures are present in some naturally fermented food products; thus, their addition to foods in a purified form should pose no risk to consumers. Although bacteriocins, such as pediocin, are probably safe, they are not yet approved as food additives. The use of bacteriocin in combination with traditional methods of preservation in close conjunction with GMPs and strict sanitation could be effective in controlling *L. monocytogenes* on fresh meats and processed meat products, especially for RTE processed products.

**Post-Packaging Thermal Pasteurization (PPTP)**

**Introduction**

Thermal treatment is a CCP in many manufactured prepared foods for controlling contamination by pathogens including *L. monocytogenes* (Mackey and Bratchell, 1989). Thermal processing is one of the most
widely used means to preserve food and to destroy harmful microorganisms, thus rendering food safe for human consumption (Lou and Yousef, 1999). For example, cooking frankfurters to an internal temperature of 71°C (160°F) should kill *L. monocytogenes* if present at $10^3$ CFU/g or less. Roering et al. (1998) reported that application of thermal pasteurization is a feasible method to control *L. monocytogenes* in packaged summer sausage. PPTP treatments would be especially effective, assuming no further handling of the product after packaging. Samelis et al. (2002) suggested that PPTP treatments that immerse packaged frankfurters (1-link or 2-link packages) in hot water (80°C, 60 s) immediately reduced the inoculated population of *L. monocytogenes* by 0.4 to 0.9 log CFU/cm$^2$ after heat treatments.

**Heat is one of the most conventional, common and effective means used today**

The use of adequate heat treatment to destroy pathogenic and spoilage microorganisms is one of the most effective food-preservation processes in use today and has been used for centuries (Juneja, 2002). Heat treatment designed to achieve a specific lethality for foodborne pathogens is a critical control factor in food processing and is fundamentally important to assure the shelf-life and microbiological safety of thermally processed foods.

Assuming no further handling of the product after packaging, adequate PPTP treatments, such as heating at 77°C or greater for at least 60 s, should be sufficient to eliminate relatively low levels of *L. monocytogenes* resulting from post-processing contamination and to maintain product safety until the package is opened for consumption (Roering et al., 1998). The PPTP treatments may also provide extra benefits such as reducing levels of *Escherichia coli* O157:H7 and *Salmonella* spp. on processed meat products.

**Different types of thermal pasteurization for preservation of meat products**

**Thermal pasteurization by hot water submersion.** Cooksey et al. (1993) reported that a 4-log reduction was obtained by using PPTP treatments for fully cooked 50g beef loin chunks inoculated with *L. monocytogenes* and heated at 82.2°C to an internal temperature of 60°C. Precooked beef roasts (< 2 lb), that were surface-inoculated with *L. monocytogenes* before packaging, were sealed in cook-in bags and then pasteurized at 91°C or 96°C for either 3 or 5 min by submersion heating in hot water (Hardin et al., 1993). These authors found a 4.5-log reduction after 5-min heating time. Muriana et al. (2002) reported that higher temperatures, ranging from 90.6°C to 96.1°C, for 2 min resulted in significant reductions of *L. monocytogenes* in most RTE deli meat products. The authors suggested that this process may be an effective microbial intervention step for *L. monocytogenes*, including RTE deli-style meats with irregular surface imperfections.

In a study by Roering et al. (1998) summer sausage in single-chub packages was surface-inoculated with ca. $10^8$ CFU/ml of a 3-strain mixture of *L. monocytogenes* and vacuum-sealed. Reductions of pathogen counts of ca. 3 log CFU/g were achieved by pasteurization at 77°C, 88°C or 99°C for 90 s, 60 s or 30 s, respectively (Roering et al., 1998). These authors suggested that pasteurization temperatures at 77°C or greater for 60 s to 120 s was sufficient to eliminate an appreciable number of *L. monocytogenes* on single-chub samples in packages. Packages containing 2 frankfurters without any treatments, immersed in hot water at 75°C or 85°C
for 30 s to 90 s, provided an immediate reduction of *L. monocytogenes* counts of ca. 1 to 3 logs, from an initial inoculation population of 3.9 log CFU/g (Samelis et al., 2002).

**Steam pasteurization.** Steam surface pasteurization is another means to effectively eliminate or reduce pathogens and can be carefully designed to conserve product quality, especially for meat products with irregularly shaped surfaces (Cygnarowicz-Provost et al., 1994; Kozempel et al., 2000; Murphy and Berrang, 2002). Flash steam surface pasteurization at 115°C to 136°C for 30 s to 40 s for prepackaged beef frankfurters surfaced-inoculated with $1.02 \times 10^7$ CFU/ml *L. monocytogenes* provided a 4-log reduction in counts without severely affecting product color or weight (Cygnarowicz-Provost et al., 1994). The authors suggested that steam surface pasteurization offers promising results for maintaining high-quality, microbiologically safe, processed meat products with extended shelf-life.

Steam surface pasteurization has been recommended for possible use to pasteurize chicken or turkey frankfurters, sausages or fresh meat and poultry (Cygnarowicz-Provost et al., 1994). Irregularly shaped surfaces should not create a problem because a condensable gas is used. In another study, fully cooked chicken breast strips (13 mm wide) were surface-inoculated with *Listeria innocua* ($10^7$ CFU/g), which was developed as a heat-resistance indicator for *L. monocytogenes*. The strips were vacuum-packaged and treated with steam or hot water at 88°C for 10 to 35 min. The results showed that heating times significantly affected the survival of *L. innocua*. At 25 min, the reduction for *L. innocua* was ca. 2 logs, and at 35 min the reduction was around 7 logs. However, significant differences were found for total moisture and expressible moisture among the strips that were heat-treated at 88°C for 35 min and the untreated controls. A heating period of 35 min may be too long to maintain desirable product quality.

**Cooking in bags.** Production of “sous-vide” processed refrigerated foods, which require a long time for the temperature to rise during low-temperature heating/cooking, is a means of cooking in bags after sealing (Linton et al., 1990). However, the conditions of heating at lower temperatures and for longer heating times may induce heat shock response by microorganisms and increase subsequent heat resistance.

**Moist heating.** The effect of moist heat on populations of *L. monocytogenes* on chicken breast was investigated (Carpenter and Harrison, 1989; Harrison and Carpenter, 1989). *L. monocytogenes* survived on samples with low inoculation levels ($3.2 \times 10^2$ to $4.7 \times 10^4$ CFU/g) following cooking to an internal endpoint temperature of 73.9°C. Chicken breasts were also inoculated with $10^6$ to $10^7$ CFU/g and cooked at one of five different temperatures ranging from 65.6°C to 82.2°C. Although lethality was found to be directly proportional to the cooking temperature, *L. monocytogenes* survived at all temperatures, including 82.2°C. Numbers of *L. monocytogenes* increased significantly in all samples during storage at 4°C, except for the samples that received the highest temperature treatment (82.2°C). The authors observed that moist heat treatments were more effective in reducing numbers of *L. monocytogenes* than were dry heat treatments.

**Factors affecting inhibitory effectiveness of thermal pasteurization**

According to the results from several studies (Hardin et al., 1993; Roering et al., 1998; Muriana et al., 2002), heating temperatures at 90.6°C to 99°C effectively inactivate *L. monocytogenes* on the surface of meat.
products. It is evident that heating temperatures (77°C to 99°C) and time (60 s to 90 s) both are essential to inactivate this pathogen on summer sausage in packages (Roering et al., 1998). The data from their study showed that the reductions of *L. monocytogenes* were proportional to the immersion heating temperatures and times. But, other factors still can affect the inhibitory effectiveness of thermal pasteurization.

**Heating temperatures.** The PPTP treatment may be an effective microbial intervention to dramatically eliminate *L. monocytogenes* on precooked packaged beef chunks and RTE deli-style meats (Cooksey et al., 1993; Muriana et al., 2002) and can affect other indigenous microorganisms (Cooksey et al., 1993). Muriana et al. (2002) added a mixed cocktail of four strains of *L. monocytogenes*, which was suspended in product purge, into bags packaged with a variety of large-size RTE meat products (5 to 12 lb), including turkey, ham and roast beef. On these various *L. monocytogenes*-inoculated RTE deli products, 2- to 4- log reductions were achieved when the products were submersion-heated at 90.6°C to 96.1°C for 2 to 10 min. The authors found that a minimal heating time of 2 min at 90.6°C to 96.1°C can readily provide 2-log reductions in most RTE deli meats.

Low-heat processing or pasteurization refers to the use of relatively mild heat treatment and is widely accepted as an effective means of destroying non-spore-forming pathogenic microorganisms and significantly reducing the number of natural spoilage microflora, thereby extending the shelf-life of pasteurized products (Juneja, 2002). Bacterial spores and some heat-resistant enzymes are not destroyed by this pasteurization process and limit the shelf-life of the product.

Lethality was found to be directly proportional to cooking temperatures (Harrison and Carpenter, 1989). But, *L. monocytogenes* could still be recovered from chicken breasts, which were inoculated with $10^6$ to $10^7$ CFU/g and cooked at the highest temperature (as high as 82.2°C internal temperature) used in this study. Mackey and Bratchell (1989) also agreed that *L. monocytogenes* can survive heating at 80°C.

**Heating times.** The higher the initial microbial population in a food product, the longer is the processing/heating time at a given temperature required to achieve a specific lethality of microorganisms (Juneja, 2002). It was observed that the population reductions of *L. monocytogenes* increased with increasing heating time (30 s to 240 s) at individual heating temperatures (66°C, 77°C, 88°C and 99°C) (Roering et al., 1998).

**Package types.** Samelis et al. (2002) reported that frankfurters (single-link or 2-link packages) immersed in hot water at 75°C or 85°C for 30 s to 90 s without any other treatments provided immediate reductions of *L. monocytogenes* and kept populations lower than those of the unheated controls throughout storage at 4°C. The results showed that the initial reductions were greater in frankfurter samples in single-link packages than in samples with 2-link packages. The frankfurters in single-link packages had greater antilisterial effectiveness than those in 2-link packages throughout storage at 4°C. The data showed that the samples in single-link frankfurter packages and submersed in hot water for 90 s were stored for 50 days before significant growth occurred and this growth was still lower (2.1 logs) than the initial inoculation level (3.9 logs) of the unheated controls. However, *L. monocytogenes* in packages containing 2-link frankfurters was able to recover
and grow significantly after 20 to 30 days of storage and the population was above 6 log CFU/cm² by 50 days of storage at 4°C.

**Heating rates.** Conditions similar to those that result in heat shock exist in food processing (Lou and Yousef, 1999). Slow heating or cooking, preheating, hot water, mild thermal processes, and holding food in warm trays (as occurs in food service establishments) are examples of heat shock that may occur during food processing and handling. Heat shock may result when foods are minimally processed or when the food is too bulky to allow rapid heating. Heat shock may also occur from production of “sous-vide” processed refrigerated foods, which involve a slow increase in temperature with low-temperature heating/cooking (Linton et al., 1990). The thermostolerance of *L. monocytogenes* has been found to be increased by low heating rates (Quintavalla and Campanini, 1991; Kim et al., 1994; Stephens et al., 1994). Quintavalla and Campanini (1991) found that *L. monocytogenes* became more heat resistant during slow (0.5°C/min) rather than fast heating. A nearly twofold increase in the D-values for *L. monocytogenes* was reported by Kim et al. (1994) when the pathogen was heated in ground pork at 1.3°C/min compared with 8.0°C/min.

**Surface temperature on meat products.** A special phenomenon that was observed in a series of PPTP tests was a tendency of lower pathogen reduction at 96.1°C than that obtained at 90.6°C or 93.3°C for the same product line (Muriana et al., 2002). The authors suggested that excess chilled purge, which migrated from the chilled product interior to the product surface, counteracted the heat gradient effects. Although the heating times used in this study were as long as 10 min at 96.1°C, the interior temperature of the deli-style meat products did not change. The effects of heat penetration were limited to only the outer 1-cm surface of the samples, and surface temperature equilibrated, back to below 12°C within a few minutes after immersion in a chill tank. Although heating time was extended, most of the inactivation of *L. monocytogenes* was usually obtained within 4 to 5 min, obviating the need for further heating.

**Surface imperfections of meat products in packages.** Muriana et al. (2002) indicated that *L. monocytogenes* cells (~10⁷ CFU/g) infiltrated the least-processed irregular surface areas, such as surface cuts, folds, grooves and skin, and suggested that this may be one of the major factors that shields bacteria from heat and causes the migration of chilled purge to the product surface.

**Factors affecting heat resistance of microorganisms**

Heat resistance of microorganisms varies, depending on the species and strain of bacteria, food composition, physical stage of microbial cells and recovery conditions (type of media, temperature, atmosphere and time of incubation) (Juneja, 2002). Food characteristics leading to increased heat resistance of an organism include water activity and the presence of carbohydrate, lipids, proteins and salt. Alterations in membrane fatty acid profiles result in an altered response to subsequent heat treatments. Added preservatives and heating methods also need to be considered. For example, a sublethal heat stress renders an organism more resistant to subsequent heat treatment.
Mechanisms of thermal pasteurization effects on *L. monocytogenes*

**Cellular components.** Thermal pasteurization will create changes in the macromolecules within a cell (Hoffmans et al., 1997). It has been found that ribosomal RNA was broken down when *Staphylococcus aureus* cells were heated (Allwood and Russell, 1967, as cited in Juneja, 2002). Additionally, heat injury can damage TCA-cycle enzymes, slow RNA replication, and induce DNA changes (Gray et al. 1973, as cited in Banwart, 1989). Also, Gomez and Sinskey (1973), as cited in Hoffmans (1997), showed DNA breaks to be related to heat injury of *Salmonellae*. A study by Gray et al. (1973), as cited in Banwart (1989), showed that the degradation of RNA and protein synthesis in *Pseudomonas fluorescens* could be the result of heat-induced stress. Jay (1996) noted that not all cells in a population will endure the same level of injury. Even though injury to cell membranes and ribosomes is common in sublethal heat stress, not all forms of stresses produce identifiable results.

Potential targets of heat damage include nucleic acid, proteins and enzymes, and cellular membranes (Juneja, 2002). Tomlins and Ordal (1971), as cited in Hoffmans (1997), found that ribosomal degradation during thermal injury of *Salmonella typhimurium* cells was reduced during resuscitation, and ribosomes were regenerated. Jay (1996) also noted that repair of cell injury will proceed in the general absence of cell wall and protein synthesis. In addition, repair of ribosomes and cell membrane seems to be necessary for resuscitation of cells injured by heat and irradiation. As is known, injury to pathogenic organisms eliminates their ability to cause disease, but once the cells are repaired, pathogenicity is restored (Meyer and Donnelly, 1992).

**Thermotolerance induced by stress adaptation.** Environmental stresses can induce stress adaptation or stress-protective responses by *L. monocytogenes* when the pathogen is subjected to conditions such as high or low temperatures or acidic conditions (Lou and Yousef, 1997). For example, incubating *L. monocytogenes* at a high but sublethal temperature will induce heat shock response (Lou and Yousef, 1996). Then, resistance of *L. monocytogenes* to subsequent lethal heat or other lethal factors can be greatly increased. The heat resistance of *L. monocytogenes* 5S suspended in culture medium of TSB containing 0.3% (w/v) yeast extract or in pork emulsion, and heated at temperatures of 60°C, 63°C and 66°C was determined (Quintavalla and Campanini, 1991). The results of this study indicated thermotolerance of *L. monocytogenes* in a pork emulsion was two to three times more than it was in broth.

Curing salts increased heat resistance of *L. monocytogenes* in beef samples (Mackey et al., 1990). Research on the heat resistance of *L. monocytogenes* has illustrated that the composition of the heating menstruum can dramatically affect thermal destruction of this pathogen. For example, in food systems such as ground meats, thermotolerance can be increased with the addition of salt or curing-salt mixtures (Schoeni et al., 1991; Yen et al., 1991). Juneja and Eblen (1999) revealed that NaCl concentration protected *L. monocytogenes* against the lethal effect of heat, but high sodium pyrophosphate increased the sensitivity of *L. monocytogenes*. This result was different from a study by Yen et al. (1991), in which a phosphate mixture (0.4%) protected *L. monocytogenes* from thermal destruction. Yen et al. (1991) reported that destruction of *L. monocytogenes* was 3 logs per gram less in ground pork with 3% NaCl than that for ground pork without added NaCl. The same
result was found with addition of 1% dextrose. This study also found that sodium nitrite (156 ppm) and sodium erythorbate (550 ppm) did not influence the degree of thermal destruction of *L. monocytogenes* cells in ground pork. The greatest protective effect was observed when all curing ingredients were combined and added to ground pork. Consequently, the authors concluded that the probability of *L. monocytogenes* survival during thermal processing is greater in cured than in fresh meats. This finding may provide an explanation for why *L. monocytogenes* can survive in frankfurter packages well and grow during the remainder of storage after resuscitation.

Several studies have documented the correlation between bacterial membrane fatty acid composition and heat resistance of pathogens (Beuchat and Worthington, 1976; Juneja et al., 1998). *Vibrio parahaemolyticus* grown at different temperatures and at different sodium chloride concentrations showed alterations in the membrane fatty acid profile that correlated with an altered response to subsequent heat treatment (Beuchat and Worthington, 1976). Juneja et al. (1998) demonstrated an altered heat resistance along with altered membrane fatty acid profiles in *L. monocytogenes* grown in different acidic environments at different temperatures. Heat shock also may increase the virulence of *L. monocytogenes* (Archer, 1996).

**Adaptation to environmental stresses.** Besides heat shock, adaptation to other environmental stresses may increase the thermotolerance of pathogens. Cross-over protection in which exposure to one stress alters resistance to another can also occur. For example, acid pH exposure or osmotic shock can induce stress responses, including resistance to thermal stress (Jorgensen et al., 1995). Starvation and adaptation of *L. monocytogenes* to sublethal levels of HCl, ethanol and hydrogen peroxide significantly increase the thermotolerance of the pathogen (Lou and Yousef, 1996).

**Heat resistance – heat shock proteins (HSPs).** Not only is *L. monocytogenes* able to grow at refrigeration (4°C) or lower temperatures, the pathogen also has a higher heat resistance than many other non-spore-forming foodborne pathogens and is able to survive and grow in many various environments (Brackett, 1988). Thus, inadequate thermal pasteurization can induce a protective function such as production of HSPs. Hoffmans et al. (1997) found that sublethal injury is reversible, and restoration of pathogenic potential can occur. Injured cells of *L. monocytogenes* are likely to escape detection by selective medium because current techniques are not adequate to recover injured cells.

Linton et al. (1990) investigated the effects of age of *L. monocytogenes* on thermal resistance in foods; these authors reported that cells in the stationary phase of growth show more heat tolerance than do cells in the log phase of growth. Linton et al. (1992) also found that "heat-shocked" cells (microbial cells that acquire heat resistance when exposed to mild heat stress) may be more heat tolerant than are "non-heat-shocked" cells. One plausible explanation is synthesis of HSPs, which are produced in response to heat, glucose starvation or the presence of hydrogen peroxide (Linton et al., 1990). The HSPs protect cells from subsequent heat exposure.

This response is well preserved in the genetic system found in organisms from bacteria to animals. In all organisms examined, response to elevated temperatures produced proteins encoded by the hsp70 and hsp90 gene families. The heat-shock response, where the proteins synthesized (HSPs) protect cells from harmful
effects of heat or other stresses, is stimulated by mild stress (mild heat). This response is rapid and intense, giving the effect of an emergency or survival response (Linguist and Craig, 1988, as cited in Hoffmans et al., 1997). Linton et al. (1992) reported that log-phase cells of *L. monocytogenes* Scott A were heat shocked in TSB plus 0.6% yeast extract broth at 48°C for 10 min, followed by heating at 55°C for up to 50 min. The physiological condition of the microorganism, the enumeration medium and the growth environment greatly affected the heat resistance of log-phase cells of *L. monocytogenes* Scott A (Linton et al., 1992). D55°C-value for heat shocked cells was 1.4-fold higher than for cells without heat shock (9.55 min vs. 6.69 min).

**Practical applications of PPTP for meat preservation**

**Shrink-wrapped bags or prolonging heating time.** Packaging procedures for sausage-type meat products, such as frankfurters and summer sausage, often include hot water or steam to shrink bags for better appearance. This operation provides an opportunity to perform PPTP treatments simultaneously, if 96°C hot water or steam and prolonged heating time of up to 60 s are used. Contamination of these products by *L. monocytogenes* should be significantly reduced or eliminated (Roering et al., 1998). Production of “sous-vide” processed refrigerated foods, which involve a slow increase in temperature and a low-temperature heating/cooking treatment may induce heat shock response (Linton et al., 1990). The minimal processing by mild heat or a lower heat rate for bulky foods may result in heat shock. The thermotolerance of *L. monocytogenes* can be increased by low heating rates (Quintavalla and Campanini, 1991; Kim et al., 1994; Stephens et al., 1994).

**A CCP in a HAACP plan.** Roering et al. (1998) indicated that PPTP treatments at temperatures of 77°C or greater for 60 s to 120 s was sufficient to inactivate appreciable numbers of *L. monocytogenes*. As detected by visual inspection, such processes did not cause defects, such as “greasing out” or textural changes, on the finished products. The treatments under the conditions mentioned above did not appreciably change the flavor or color of the finished product. Assuming no further handling of the product after packaging, these conditions for PPTP treatments should be sufficient to inactivate the relatively low contamination levels of *L. monocytogenes* resulting from post-processing contamination. PPTP treatments may also prove useful for reducing levels of other pathogens (Roering et al., 1998).

The findings from a study by Muriana et al. (2002) have been accepted as confirmatory data for several companies who have applied PPTP treatments as a CCP in their HACCP plans. The authors asserted that the PPTP treatment is one of several technologies that could be implemented within the hurdle concept to be a CCP for reduction of incidental surface *L. monocytogenes* contamination on RTE deli meat products.

**Practical applications of PPTP combined with other barriers in the meat industry**

It is well known that pathogen problems typically develop from post-thermal processing contamination because *L. monocytogenes* is relatively heat-susceptible. Samelis et al. (2001) suggested that PPTP treatments may assist in increasing the antilisterial effects of post-processing application of antimicrobials, such as pediocin, in cooked cured meats (Roering et al., 1998). A 1- to 2-log reduction in surface contaminants of commercially manufactured products may be adequate to completely inactivate or reduce *L. monocytogenes*
populations to undetectable levels. Such low levels of heat-stressed cells may be unable to recover and may be
drastic heat treatments. Such low levels of heat-stressed cells may be unable to recover and may be
more susceptible to antimicrobials (Samelis et al., 2002).

**Combinations of bacteriocin and heat.** Severe heat treatment can negatively affect the organoleptic
properties and nutritional values of foods (Juneja, 2002). To avoid the undesirable effects of heat, one approach
is to use heat in combination with other known preservation techniques. The use of combination treatments
incorporating mild heat can result in enhanced preservative action by having an additive or synergistic effect on
microbial inactivation and/or reduce the severity of one or all the treatments.

Addition of various concentrations of the bacteriocin nisin renders bacteria sensitive to the lethal
effects of heat, thereby enhancing the effectiveness of thermal processes during mild heat treatments. Nisin was
added at a level of 25 mg/kg to the brine surrounding canned lobster and subjected to a heat process resulting in
internal temperatures of 60°C for 5 min and 65°C for 2 min (Budu-Amoako et al., 1999). The combinations of
nisin and heat provided a 3- to 5-log reduction of *L. monocytogenes* compared to a 1- to 3-log reduction for heat
or nisin alone. Henning et al. (1986) indicated that sublethal heat increases the permeability of the cell to allow
bacteriocins, such as nisin, much easier access to the cytoplasmic membrane.

The effects and interactions of temperature, pH, sodium chloride content and sodium pyrophosphate
concentration are among the variables that researchers have considered when attempting to assess the heat-
inactivation kinetics of foodborne pathogens. Incorporation of these multiple barriers can increase the
sensitivity of cells/spores to heat, thereby reducing heat requirements and ensuring the safety of RTE food
products (Juneja, 2002).

**Combination treatments of pediocin and PPTP.** Pediocin (Bhuniya et al., 1991) and PPTP treatments
(Roering et al., 1998) have been reported individually to have significant benefits for control of post-processing
contamination of frankfurters by *L. monocytogenes*. Treatments, such as spraying or dipping of products in
antimicrobial solutions before packaging (Samelis et al., 2001) can be combined with PPTP treatments (Roering
et al., 1998) to enhance effectiveness. Results from a study by Samelis et al. (2002) indicated that the limited
effectiveness of PPTP against *L. monocytogenes* in the absence of antimicrobials may be of practical
significance. Consequently, meat plants should not rely solely on this method without previous evaluation of its
effectiveness with different products.

**Post-Packaging Irradiation Pasteurization**

**Introduction**

Olson (1998) reported that the Food and Drug Administration (FDA) approved irradiation for red meat
in December 1997. The author indicated that appropriate absorbed doses of radiation effectively kill disease-
causing bacteria and delay food spoilage. Ionizing radiation possesses enough energy to ionize molecules in its
path and can inactivate foodborne microorganisms without increasing the temperature of the irradiated food
(Mendonca, 2002).
It has been estimated that 523 deaths occur each year that involve, in part, foodborne illness by pathogens (Todd, 1989). Between 1973 and 1987, the spectrum of diseases caused by foodborne pathogens broadened, including *L. monocytogenes* (Bean and Griffin, 1990). In this 15-year period (with 7,458 outbreaks and 237,545 cases), bacterial pathogens accounted for 90% of the deaths, with *L. monocytogenes* having the highest death-to-case ratio of 317 deaths to 1,000 cases. Preliminary estimates show that for the United States, the full economic impact of foodborne diseases is about 12.6 million cases costing $8.4 billion (Todd, 1989). This estimate includes the cost from illness, death and business losses. *L. monocytogenes* accounts for the third highest overall cost ($3.3 million) among all bacterial foodborne diseases. The average cost per case of *L. monocytogenes* was listed at $12,520 (Todd, 1989). The growing awareness of foodborne diseases caused by microorganisms has greatly increased interest in the use of food irradiation for destroying foodborne pathogens (Mendonca, 2002).

**Different types of irradiation for preservation of meat products**

**Gamma irradiation.** Some investigations have concluded that a gamma irradiation dose of 2.0 kGy would be adequate to reduce the numbers of *L. monocytogenes* by 4 logs (Huhtanen et al., 1989). It was found that irradiation of a chicken meat slurry and of raw ground beef at 2.5 kGy prior to refrigeration was an efficient way of preserving meat products contaminated with *L. monocytogenes* at $10^3$ to $10^4$ CFU/g (Gursel and Gurakan, 1997).

**Ionizing irradiation.** More recent studies involving the use of electron beam irradiation for improving the microbial safety of foods have demonstrated the efficiency of this irradiation type for inactivating *L. monocytogenes*. Medium doses (1.8 to 2.0 kGy) of electron beam irradiation reduced the populations of *L. monocytogenes* in cooked pork chops and cured hams to undetectable levels (Fu et al., 1995b). Tarte et al. (1996) investigated the sensitivity of three strains of *L. monocytogenes* along with *L. innocua* and *L. ivanovii* in ground pork treated with electron beam irradiation. Ground pork was inoculated with one of five strains of *Listeria* and irradiated from 0 to 1.25 kGy at 0.25 kGy intervals. The authors concluded that the dose range (1.5 to 4.5 kGy) being considered by the FDA for irradiation of fresh beef and pork was sufficient for eliminating *L. monocytogenes* in pork.

**Advantages and disadvantages**

Irradiation of food can effectively reduce or eliminate foodborne pathogens and spoilage microorganisms while maintaining wholesomeness and sensory quality (Olson, 1998). The author suggested that selection of appropriate treatment conditions can minimize or prevent objectionable quality changes in food. Irradiation of turkey frankfurters with 5 to 10 kGy doses at two temperatures (2°C and -30°C) generally resulted in no change in product tenderness, freshness, off-flavor or overall acceptability (Barbut et al., 1988). It is well known that many food processes, such as cooking, change nutrient content much more than does irradiation (Murano, 1995a). Trace elements and minerals are not affected by irradiation. Macronutrients, such as protein, carbonates and fat, are also not affected by doses up to 10 kGy.
Sundaram (1999) employed different treatment combinations using pediocin combined with irradiation (3 kGy) and high hydrostatic pressure (HHP) (75,00 psi for 5 min) as well as other treatments to extend the shelf-life of raw and cooked lean beefsteaks. This study indicated that pediocin is tolerant to stresses of irradiation and HHP at certain levels. It implied that irradiation does not change structure or functionality of pediocin so that combinations of these treatments can be used to inhibit the growth of *L. monocytogenes* or the other gram-positive bacteria.

Disadvantages to irradiation are that some vitamins in foods may be sensitive to irradiation processing (Murano, 1995b). Thiamin and ascorbic acid (vitamin C), which are water-soluble vitamins, are the most likely to be affected by irradiation. Thiamin loss may be due to indirect effects caused by free radical reactions. Of the fat-soluble vitamins, only vitamins A and E are affected to some degree by irradiation. Irradiation can cause oxidation of myoglobin and fat, and may result in discoloration and rancidity or production of off-odors or off-flavor of fresh meat and poultry (Murano, 1995b; Lee et al., 1996). However, while irradiation may accelerate lipid oxidation, addition of high levels of tocopherol in pork products can improve this situation (Jo and Ahn, 2000). A potential concern for the application of antioxidants, such as ascorbic acid, citric acid and erythorbate, is that they may increase the irradiation resistance of *L. monocytogenes* (Sommers and Thayer, 2000). A 0.1% or greater antioxidant solution of sodium erythorbate increased the irradiation resistance of *L. monocytogenes* (Sommers et al., 2002). However, this antioxidant, applied on the surface of cured meats, is probably not present in sufficient amounts to protect *L. monocytogenes* against the lethal effects of ionizing irradiation. Therefore, the industrial practice of applying antioxidants, such as sodium ascorbate or erythorbate, on the surfaces of cured meat products should not compromise the efficiency of irradiation as an antimicrobial approach.

**Consumer acceptance**

Irradiation of raw meat and poultry is currently allowed to control foodborne pathogenic microorganisms and retailers began marketing irradiated beef products in mid-2000 (Frenzen et al., 2001). The 1998-1999 FoodNet Population Survey which covered 11% of the U.S. population, indicated that nearly one-half (49.8%) of the 10,780 adult respondents were willing to purchase irradiated meat or poultry, but the cost of irradiated products was not considered in this study (Frenzen et al., 2001). After adjusting for other factors, consumer acceptance of irradiated products was associated with male gender, greater education, higher household income, food irradiation knowledge, household exposure to raw meat and poultry, consumption of animal flesh and geographic location. It is still unclear why persons at higher risk of foodborne illness were not more willing to buy irradiated products, which could reduce the hazards they faced from handling or undercooking raw meat or poultry contaminated by foodborne pathogenic microorganisms. Market simulation experiments have found that the proportion of consumers purchasing irradiated meat and poultry increased after study participants received additional information about food irradiation (Hashim et al., 1995). These findings suggested that targeted educational messages about food irradiation could increase consumer acceptance of irradiated food (Bruhn, 1995).
Benefits for the meat industry

Thayer (1990) indicated that a nonsterilizing food preservation technology for red meat, poultry or other meats is the major benefit of ionizing irradiation from a safety standpoint. Irradiation provides an approach to reduce use of chemical additives because it decreases the microbial load and eliminates some foodborne pathogens. Irradiation also decreases the opportunity for post-processing contamination if products are packaged before being irradiated (Fu et al., 1995b). These authors found that irradiation was effective for reducing *L. monocytogenes* on cured ham, especially at medium doses (2.0 kGy). Accompanied by refrigeration, irradiation should ensure the safety of these products and should extend their shelf-life.

Irradiation at appropriate doses was also more effective in combination with the salt and nitrite added for bacterial control in cured ham. Radomyski et al. (1994) suggested that irradiation at doses up to 3.0 kGy are sufficient to eliminate most pathogens in meats.

Food irradiation provides several benefits for consumers as well as the meat industry. Irradiation improves the safety of fresh meats by reducing or eliminating foodborne pathogenic microorganisms and extends the shelf-life of these products during refrigerated storage. Additionally, scientific studies have shown that these benefits can be achieved without adverse effects on product quality (Murano, 1995b). The safety of irradiated meats has been established by extensive research. Consumers who choose to eat irradiated raw meat and poultry as a substitute for non-irradiated products can reduce their risk of foodborne illness, and those at increased risk should experience the greatest health benefits.

Factors affecting survival and growth of *L. monocytogenes*

Inactivation of foodborne microorganisms by ionizing radiation is influenced by several factors. These factors include irradiation dose, numbers and types of microorganisms, food composition and preservation method, temperature and atmospheric gas composition (Thayer, 1990; Mendonca, 2002). The irradiation dose applied to a food is the most important factor of the irradiation process. In general, higher doses of ionizing radiation cause greater inactivation of microorganisms.

Microbial numbers have the same impact on the effectiveness of irradiation as does use of certain other effective food preservation methods (Mendonca, 2002). Large populations of microorganisms reduce the inhibitory effectiveness at given irradiation doses. It was observed that gram-negative bacteria are more sensitive to irradiation than are gram-positive bacteria (Monk et al., 1995; Jay, 1996; Mendonca, 2002). Irradiation doses of at least 1.0 kGy have been demonstrated to virtually eliminate gram-negative bacteria in food but have a much lower effect on gram-positive, lactic acid-producing bacteria (Ehioba et al., 1988; Lambert et al., 1992; Thayer et al., 1993). Non-spore-formers are more sensitive than are spore-formers. With respect to the physiological state of bacteria, lag phase or stationary phase cells tend to be less sensitive to irradiation than do log phase cells.

Mendonca (2002) reported that increased amounts of protein in foods tend to provide a protective effect for bacteria during irradiation processing. Proteins and other food components, including natural antioxidants such as vitamin C and vitamin E, compete for free radicals from activated molecules and the
radiolysis of water. The competition for free radicals reduces the antimicrobial effect of irradiation. Food preservation methods, such as heating, acidification, high hydrostatic pressure and addition of chemical food preservatives, increase the sensitivity of microorganisms to ionizing radiation by decreasing the number of survivors.

Although differences between \textit{L. monocytogenes} strains likely account for most of the observed variation in D-values, radiation sensitivity of \textit{L. monocytogenes} is also affected by age of culture, irradiation menstruum, and the type of medium used to enumerate the pathogen after irradiation. According to Huhtanen et al. (1989), 1.5- and 2.5-h-old cultures of \textit{L. monocytogenes} were somewhat more resistant to gamma radiation than were those incubated 5 and 18 h before exposure. It was not surprising that \textit{L. monocytogenes} is more resistant to gamma radiation when present in foods than in culture media, especially in nonselective agar, such as tryptic soy agar plus 0.6% yeast extract (TSA-YE). Patterson (1989) reported that the D-value for radiation resistance is markedly affected by the type of plating media used to enumerate the pathogen after irradiation. A significantly higher (P < 0.05) D-value resulted from increased recovery of the pathogen with nonselective rather than highly selective plating media. These findings indicate that substantial numbers of \textit{Listeriae} were sublethally injured during exposure to gamma irradiation. Repair and subsequent growth of injured cells are frequently inhibited by some of the selective agents used in highly selective media.

**Antilisterial effects of irradiation**

Adequate absorbed doses of irradiation effectively kill disease-causing pathogens, including \textit{L. monocytogenes}, and delay food spoilage (Olson, 1998). Fu et al. (1995b) indicated that irradiation provides a method to reduce chemical additives because it decreases the load of microorganisms and eliminates some foodborne pathogens. Low-dose to medium-dose (≤ 3.0 kGy) irradiation has become available to control pathogens in meat products (Radomyski et al., 1994; Lee et al., 1996) and offers the potential to minimize changes in the quality of finished product characteristics (Radomyski et al., 1994). Lebepe et al. (1990) reported that vacuum-packaged pork loins, gamma-irradiated at 3.0 kGy and stored at 2°C to 4°C, extended the microbiological shelf-life to over 90 days compared to 41 days for nonirradiated samples. Fu et al. (1995b) reported that irradiation with medium doses of 1.5 kGy for steaks and 2.0 kGy for ground beef achieved 3- and 5-log reductions of pathogen populations, respectively. Storage at 7°C was effective in this study for suppressing the growth of \textit{L. monocytogenes} in all samples, with no increasing numbers observed during storage.

Fu et al. (1995b) also showed that irradiation was effective in reducing \textit{L. monocytogenes} on inoculated ham. The authors found that medium-dose irradiation of 2.0 kGy resulted in a greater population reduction of this pathogen than 0.9 kGy, but, did not eliminate the pathogen because some cells were able to recover when temperature was elevated to 25°C. The final populations on pork chops and ham after storage for 9 days was less than those on control samples or on samples exposed to low-dose irradiation (0.75 to 0.9 kGy). The authors also observed that storage at 7°C contributed to inhibition of growth; the numbers of the pathogen only increased 1 log on both nonirradiated and irradiated (0.9 kGy) ham. They suggested that addition of nitrite
to ham may be responsible for the greater inhibition of *L. monocytogenes* than observed for pork chops, especially when low-dose irradiation was employed. Radomyski et al. (1993) indicated that food irradiation should be considered as a major technology to meet the demands of consumers for present and future meat and poultry markets.

**Mechanisms of irradiation inhibition on *L. monocytogenes***

When microorganisms are exposed to ionizing radiation, high-energy rays and particles collide with components of the microbial cells and produce change at both molecular and atomic levels (Mendonca, 2002). Changes at the molecular level occur when collisions produce enough energy to break chemical bonds between atoms, resulting in the production of free radicals. Changes at the atomic level occur when the energy from collisions between ionizing radiation and cellular components are enough to cause an electron to be expelled from an atomic orbit producing ion pairs. The production of free radicals and ion pairs and reaction of free radicals with components of the microbial cell is the mechanism by which irradiation inactivates microorganisms.

When food is irradiated, the high-energy electrons react with various food molecules (especially water) to generate free radicals that are highly reactive. Free radicals cause extensive damage to cellular components by reacting with them. The damage to cells includes deoxyribonucleic acid (DNA) strand breaks, base damage, denaturation of proteins (Sundaram, 1999) and cytoplasmic membranes (Mendonca, 2002). Breaking bonds in DNA results in the loss of a cell’s ability to replicate. A relatively small change in the DNA of a bacterium can destroy the cell. The destruction caused by disruption of the genetic material in a living cell is the major effect of irradiation of food (Murano, 1995a). The inability of microorganisms to repair lesions caused by free radicals and other reactive species results in the loss of their ability to replicate and reproduce, which leads to cell death (Mendonca, 2002).

The direct effect on DNA involves removal of electrons from DNA molecules resulting in damage to this vital genetic material (Mendonca, 2002). High-energy rays and particles make contact with DNA and other cell components much like a bullet that hits a target. However, direct damage to vital cell components does not need to occur for irradiation to inactivate microorganisms. Microbial inactivation may result from the indirect action of irradiation involving the radiolysis of water in the cell as well as in the suspending medium. During the radiolysis of water, water molecules are altered to produce highly reactive hydrogen and hydroxyl radicals. These radicals can alter bases such as thymine to form dihydroxydihydrothymine. In addition, they can cause oxidation, reduction and breakage of carbon-to-carbon bonds of cell components, including DNA. Radiation damage to the DNA of microorganisms includes both single-strand and double-strand breaks. Free radicals can also react with each other, with dissolved oxygen and with other molecules and ions that may be present in water to form toxic oxygen derivatives and other reaction species that are lethal to cells.

A study by Sundaram (1999) suggested synergistic effects between pediocin and irradiation treatments. These effects could be explained by the following inferences. The cell membrane of *L. monocytogenes* was damaged by pediocin, with severe enough damage or lysis to cause the membrane to lose its integrity; thus, the
cells of *L. monocytogenes* were seriously injured and stopped multiplying. The cells may be unable to repair the membrane damage after addition of pediocin. The high-energy electrons in an electronic beam may then more easily penetrate cells to directly damage DNA molecules. This action would effectively damage DNA molecules to make the cells less likely to repair themselves than following treatment with pediocin or irradiation alone.

**Multiple hurdle preservation approaches**

The hurdle concept emphasizes the combined use of antimicrobial factors to inhibit growth or eliminate microorganisms from food (Lou and Yousef, 1999). When preservation factors (hurdles) are combined, an additive antimicrobial effect often is observed (Buchanan et al., 1989). Combined hurdles sometimes act synergistically to enhance microbial inhibition and inactivation beyond the additive effect. The authors suggested that there can be a synergistic interaction between two hurdles or among multiple hurdles.

Inhibition of *L. monocytogenes* by multiple hurdles was studied by investigating treatment combinations of incubation temperatures (5°C to 37°C), initial pH (6.0 to 7.5), sodium chloride (0.5% vs. 4.5%), sodium nitrite (0 to 1,000 ppm) and atmosphere (aerobic vs. anaerobic) on growth of *L. monocytogenes* in trypticase soy broth. The authors found that sodium nitrite was the most listeristatic among these five interacting variables, which affected lag phase, generation time and maximum populations. Nitrite was most effective when used in conjunction with low pH, increased sodium chloride, refrigeration temperatures and anaerobic conditions that simulated vacuum-packaging.

According to Lou and Yousef (1996, 1997), adaptation of *L. monocytogenes* to sublethal levels of acid, ethanol, hydrogen peroxide and starvation increased resistance of *L. monocytogenes* to these factors when combined with heat. This stress adaptation affects the hurdle concept because such hurdles in foods can be applied simultaneously or sequentially (Lou and Yousef, 1999). When applied sequentially, hurdles may not deliver the desired combined effect. Stress adaptation to the first encountered hurdle, which hardens pathogens and increases their resistance to subsequent preservation factors, may counteract hurdle treatments. Interaction between hurdles becomes much more complicated when the history of *Listeria* cells to be inactivated by the multiple hurdles is considered. Adaptation of *L. monocytogenes* during sublethal exposure to various preservation techniques (or stress) may protect the pathogen against subsequent exposure to the same, different, or any combination of stresses at normally lethal levels.

The formula used for frankfurters from different manufacturers may affect irradiation D-values for *L. monocytogenes* (Sommers and Thayer, 2000). If low doses of irradiation were to be used for control of *L. monocytogenes* on frankfurters, the irradiation doses should be modified by individual product formulation. The authors suggested that different brands of frankfurters may require doses from 2.45 to 3.55 kGy to achieve a 5-log reduction of *L. monocytogenes* (initial inoculation level of 3.5 x 10^8 CFU/cm^2). These differences may be due to the different ingredients used in various formulations.

Populations of *L. monocytogenes* on cooked pork chops and cured hams were reduced to 2 logs or less by low-dose (0.6 to 0.8 kGy) irradiation, but medium-dose (1.8 to 2.0 kGy) electron beam irradiation achieved
undetectable levels (Fu et al., 1995b). These authors suggested that a higher salt concentration and addition of nitrite to ham, vs. pumped chops, probably was responsible for the greater inhibition of *L. monocytogenes* in ham, especially when low-dose irradiation was applied. Buchanan et al. (1989) concluded that nitrite could have significant bacteriostatic effects on *L. monocytogenes*, particularly if used in combination with low pH (pH 6.0 or below) (Sorrels et al., 1989), vacuum packaging, high salt concentrations and adequate refrigeration.

Fu et al. (1995b) simulated mishandling of products after purchase by temperature abuse at 25°C for 2 days after hams were stored at 7°C for 7 days. The results showed that temperature abuse accelerated the recovery of injured cells of *L. monocytogenes* on ham, even though *L. monocytogenes* on the samples was reduced to virtually undetectable levels (initial inoculation level of 6 log CFU/g) after irradiation with 2.0 kGy.

**Good Manufacturing Practices (GMPs) as prerequisites for *L. monocytogenes* control**

Because *L. monocytogenes* will continue to be introduced into a plant’s environment, control must be directed toward preventing establishment and growth in the environment (Tompkin et al., 1999; Tompkin, 2002). These authors suggested that GMPs, sanitation and training targeted toward specific control of *L. monocytogenes* are prerequisite programs for the foundation of control of this pathogen. Nielsen et al. (1990) suggested that GMPs and strict sanitation need to be incorporated with traditional methods of prevention to be effective in controlling *L. monocytogenes* on fresh meat, thus assuring a safer product for consumption or further processing.

**Practical applications in a HACCP plan**

Tompkin (2002) emphasized that post-processing contamination is not controlled through the HACCP plan but throughout a broad variety of prerequisite programs. The author also indicated that *L. monocytogenes* can be controlled but not eliminated from the cooked-product processing environment. Hence, the results from several studies have suggested that post-packaging pasteurization and additional treatments, such as bacteriocins, may provide necessary incremental protection (Tompkin, 2002). Post-packaging thermal pasteurization or irradiation, which effectively inactivate *L. monocytogenes*, could be included in a HACCP plan as the last hurdle to reduce risk (Thayer, 1995; Muriana et al., 2002) of *L. monocytogenes* on processed meats.
CHAPTER 3. USE OF PEDIOCIN FOR CONTROL OF LISTERIA MONOCYTOGENES ON FRANKFURTERS

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Abstract: The effectiveness of pediocin (in ALT A™ 2341) for controlling Listeria monocytogenes on frankfurters was evaluated. Frankfurters were surfaced-inoculated with a 5-strain mixture of L. monocytogenes (3.40 or 5.20 log CFU/g) after treatments with 3,000 AU or 6,000 AU of pediocin per link, and vacuum-packaged (single-link, 5-link or 10-link packages). Treatments were evaluated for L. monocytogenes counts initially and during storage (4°C, 10°C and 25°C) for up to 12 weeks. The populations of L. monocytogenes were reduced by addition of pediocin; but were unaffected by pediocin concentrations or package types. Treatments (4°C) with 6,000 AU of pediocin effectively prolonged the time prior to growth of L. monocytogenes for 7 weeks and reduced the growth of L. monocytogenes for up to 12 weeks.

The inhibitory effectiveness of delivery systems for pediocin, including direct spray, a co-extruded casing with pediocin, and conventional casings with interior coatings of pediocin or pediocin and diacetate were also compared. Spray application was most effective. Co-extruded casings with pediocin and cellulose casings lined with pediocin showed a small, but statistically insignificant reduction of L. monocytogenes numbers.

Further work is needed to improve the different delivery systems for pediocin on frankfurters.

Keywords: Listeria monocytogenes, pediocin, frankfurter, package type, storage temperature

Introduction

Several well-publicized outbreaks of Listeria infections have been linked to ready-to-eat (RTE) processed meat products such as frankfurters (Farber and Peterkin, 1991; CDC, 1998; CDC, 1999a; CDC, 1999b). The case of Bil Mar Foods, in 1998 to 1999, was one of the most widely publicized and one which resulted in a very large product recall (CDC, 1999a). The Center for Disease Control and Prevention (CDC) reported that L. monocytogenes serotypes 4b and 4c have been isolated from frankfurters, deli meats (100 cases, 21 deaths, 22 states) (CDC, 1999b), and deli turkey meat (29 cases, 4 deaths, 3 miscarriages/stillbirths, 10 states) (CDC, 2000). It is imperative that the industry develop effective controls for L. monocytogenes, especially for RTE processed meats.

Refrigeration temperatures and vacuum-packaging are two of the most common techniques in the meat industry to inhibit the growth of most microorganisms, but these techniques do not inhibit L. monocytogenes (Glass and Doyle, 1989). The ability to grow at temperatures as low as -0.1°C to -0.4°C (Glass and Doyle,
1989; Walker et al., 1990) and the ubiquitous character of *L. monocytogenes* (Fenlon et al., 1996; Samelis and Metaxopoulos, 1999; Tompkin, 2002) allow this organism to contaminate RTE foods in the environment of processing plants. Contamination can occur after thermal processing and the pathogen can subsequently survive for long periods of time in different environments. For example, the peeling procedure for frankfurters has been proposed as a point where the contamination of this pathogen easily occurs. It has been suggested that the peeling operation should be considered a critical control point (CCP) in the Hazard Analysis and Critical Control Point (HACCP) program to reduce contamination (Wenger et al., 1990).

Sporadic contamination by pathogens may occur at slaughter and processing facilities (Fu et al., 1995). Even when Good Manufacturing Practices (GMPs) and HACCP systems are emphasized, pathogens can still contaminate meat products, survive, grow and cause disease (Tompkin, 1983; Karr et al., 1994). It is clear that additional antilisterial measures are necessary to ensure complete elimination of this pathogen from RTE meats. Post-packaging intervention strategies, such as addition of pediocin or organic acids, thermal pasteurization, irradiation pasteurization or high-pressure treatments are examples of methods that could be used to inactivate this pathogen on RTE meat products. Pediocin, is a bacteriocin that has been found to inhibit several gram-positive bacteria including *L. monocytogenes*. For example, pediocin AcH has been reported to reduce or inhibit the growth of *L. monocytogenes* (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995). This bacteriocin has been studied in a variety of meats (Nielsen et al., 1990; Ming et al., 1997; Murray and Richard, 1997) and processed meat products, including frankfurters (Berry et al., 1991), wieners (Degnan et al., 1992), and wiener exudates (Yousef et al., 1991). ALTA™ 2341, a commercially available multifunctional food ingredient, possessed intrinsic listericidal activity similar to pediocin (Schlyter et al., 1993a; Rozum and Maurer, 1997).

The use of pediocin as a surface-treatment intervention method for frankfurters may offer a means for improved control of *L. monocytogenes* on this product. Therefore, objectives of this study were to assess the effectiveness of pediocin for control of *L. monocytogenes* on the surface of RTE meat products such as frankfurters, and to evaluate the quality of frankfurters subjected to antimicrobial treatments.

Moreover, this study also evaluated the application of these antimicrobial treatments to meat products by different delivery methods including spraying, co-extrusion of casings with antimicrobials and use of casings treated with pediocin and other preservatives.

**Materials and Methods**

The experimental protocol was designed to first compare the effects of pediocin concentrations on inhibition of *L. monocytogenes* on frankfurters in different package types. Pediocin was subsequently evaluated for inhibitory effects during storage at 4°C, 10°C and 25°C. The potential to deliver pediocin to product surfaces by spraying, using pretreated casings or a casing co-extruded with pediocin was also evaluated. Finally, the treatments with pediocin were assessed for product quality changes.
Frankfurter preparation

Frankfurters were manufactured from frozen beef trim (-80% lean) and fresh pork trim (-50% lean) purchased from commercial suppliers. Frozen beef trim was removed from the freezer (-20°C) 24 h before processing and tempered at 2°C to 4°C. All trimmings were coarse ground (0.95-cm plate) and the fat content measured by Anyl-Ray (Kartridg-Pak Co., Davenport, IA). Frankfurter batches were formulated with a 45.4 kg meat block, including 22.7 kg of beef trim and 22.7 kg of pork trim, plus 9.1 kg of ice/water, 1.49 kg of spice mixture (A.C. Legg Packing Co., Inc., Birmingham, AL), 112 g of curing salt (A.C. Legg Packing Co., Inc., Birmingham, AL) and 908 g of salt.

The lean beef trim was chopped first with ice/water, salt, spices and cure to about 4.5°C after which the fat pork trim was added and chopping continued to 13.9°C to form the batter. The frankfurter batter was vacuum-stuffed (Risco Model RS 4003-165; Stoughton, MA) into 24 mm cellulose casings and linked at 12 to 14 cm in length. Stuffed frankfurters were smoked, cooked and showered in an Alkar oven (Alkar, Lodi, WI). Chilling was achieved in a 2°C cooler overnight before peeling. Finished, peeled frankfurters were stored at 2°C for 12 to 18 h before assignment to different experimental treatments. Finished weight of the frankfurters was 45 ± 5 g per link.

Fat, moisture (AOAC, 1990) and protein content (AOAC, 1993) of the finished products were measured. The results indicated that the frankfurters contained 28.25% crude fat, 53.66% moisture and 13.44% protein.

Inoculation and packaging

Frankfurters were packaged in vacuum bags using three different package types as follows: 1) 10 links per package in a double row of 5 each, 2) 5 links per package in a single row, and 3) singly packaged links. Each packaging group was divided into two subgroups for addition of one of two levels of pediocin (ALTA™ 2341, Quest Int., Sarasota, FL) before inoculation with L. monocytogenes. ALTA™ 2341 was formulated at 40% (wt/vol) in sterile, distilled water to achieve 3,000 arbitrary units (AU) of pediocin per ml. The frankfurters were placed in bags, then sprayed with either 1 or 2 ml per link of 40% ALTA™ 2341 suspension, equivalent to 3,000 AU or 6,000 AU of pediocin per link (Pdn-3000 or Pdn-6000). The vacuum bags (Cryovac® B-540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100°F, 100% RH /100 sq. in. /24 h; oxygen transmission rate = 3-6 CC at 40°F /m² /24 h /0% RH) with frankfurters and pediocin were hand-massaged for 5 s to 10 s for even distribution of pediocin, before vacuum-sealing (Multivac A 300/52, Mutivav Sepp Haggenmuller GmbH & Co., Wolfertschwenden, Germany).

For inoculation of the frankfurters, a 5-strain of cocktail mixture of L. monocytogenes cultures, including L. monocytogenes Scott A, H7764 1/2a, H7969 4b, H7962 4b and H7762 4b was used. With exception of the Scott A strain, all strains were obtained from the CDC as clinical isolates from the Bil Mar Foods outbreak of 1998-1999 (CDC, Atlanta, GA). The L. monocytogenes cultures were individually grown in trypticase soy broth containing 0.6% yeast extract (TSB-YE broth) (Difco, Becton Dickinson and Company,
Sparks, MD) at 35°C for 24 h. Then, 1 ml of culture from each individual strain was combined to give a 5-ml mixed culture of *L. monocytogenes*. The mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35°C for 24 h to reach the stationary phase. The final concentration of the original 5-strain mixture of *L. monocytogenes* was 9.30 log CFU/ml.

Frankfurters were surface-inoculated with the 5-strain mixture of *L. monocytogenes* by adding 1 ml per link of the original mixture (9.30 log CFU/ml) to result in a “high” inoculation level. The original mixture was then diluted 1:100 (7.30 log CFU/ml) to result in a “low” inoculum when 1 ml was used. The inoculum was added to the frankfurters after they were in the appropriate package and before the package was sealed. Inoculated frankfurters without pediocin served as controls and were used to determine the recovery of *L. monocytogenes*.

After inoculation, the packages were hand-massaged 5-10 s to evenly distribute the 5-strain mixture of *L. monocytogenes* on the surface of each frankfurter. After vacuum-packaging, all frankfurters were placed in cardboard boxes and stored at 2°C to 4°C for 14-18 h before sampling. Average recovery of *L. monocytogenes* from untreated samples was 3.40 and 5.20 log CFU/g for the low and high inoculation levels, respectively.

**Initial evaluation of treatments**

Survival of *L. monocytogenes* that was surface-inoculated (3.40 or 5.20 log CFU/g) on frankfurters treated with pediocin (Pdn-3000 or Pdn-6000), was determined by enumerating the surviving organisms. The inhibitory effectiveness of the treatment combinations was compared to determine the most effective combinations.

**Evaluations during storage**

Based on initial evaluation of the treatments, those treatments that showed the greatest immediate inhibitory effectiveness were selected for subsequent evaluation of long-term effectiveness during storage. Only samples containing 5 links per package were used for evaluations during storage. Frankfurter samples with the low inoculation level (ca. 3.40 log CFU/g) were used for the evaluations during storage. Samples were stored at 4°C, 10°C and 25°C for up to 12 weeks.

**Microbiological analysis**

**Initial evaluation of treatments.** Packages with frankfurters were aseptically opened, using sterile scissors, 18 h after treatments. Except for single-link packages, sampling was done by collecting two frankfurters, one from the center of the package row and one from the outside. For packages containing 10 links, the two frankfurters were taken from different rows. Frankfurters were aseptically cut in half (ca. 20-23 g portions) with sterile scissors and tweezers, and homogenized (Seward Stomacher blender, Model 4000, Tekmar™ Co., Cincinnati, OH) for two minutes in sterile stomacher bags (Whirl-Pak Filter Bag B01318, A Nasco, Ft. Atkinson, WI) with sufficient 0.1% sterile peptone water to give a 1:5 dilution of the sample. *L. monocytogenes* cells were enumerated by serially diluting 1 ml of the blended samples in 9 ml of 0.1% peptone water. Aliquots of appropriate serial dilutions were surfaced-plated on Modified Oxford (MOX) (Difco) agar with 0.1% Oxford Antimicrobial Supplement sterile solution. Samples were also plated on trypticase soy agar.
All inoculated agar plates were incubated at 35°C for 48 h. Typical colonies were enumerated, identified by gram stain and confirmed using API Listeria kits (bioMerieux, Inc., Hazelwood, MO).

**Evaluations during storage.** For enumeration of samples held in storage, packages were opened aseptically and whole frankfurters were mixed for 1 min at normal speed in a Seward Stomacher blender with an equal amount of 0.1% peptone water (50% dilution) to rinse them thoroughly. L. monocytogenes cells were enumerated by serially diluting the 1 ml rinse in 9 ml of 0.1% peptone water and plating on MOX agar and TSA plus 0.6 % yeast extract (TSA-YE) with identification as described previously. When increasing sensitivity was required, 1.0 ml samples of rinse were plated directly onto MOX or TSA-YE.

**Product quality analyses**

After completion of the microbiological evaluations, the treatments that had the most impact on L. monocytogenes were also evaluated for product quality changes. Frankfurters (5 links per package) were prepared, processed and treated in the same way as for the inoculation challenge, but without the L. monocytogenes inoculation. Quality evaluations included measurement of purge, color, texture, odor quality, pH and thiobarbituric acid (TBA) values.

**Physical and chemical analyses of frankfurters**

For purge accumulation (%), two packages were each weighed, opened and the frankfurters removed. The package and links were wiped dry and reweighed. The weight difference was calculated as purge and expressed as a percentage of unpackaged product weight (Bloukas et al., 1997).

Color (L*, a*, b*) measurements of the frankfurters were performed using a Hunter Labscan spectrophotometer (Hunter Associated Laboratories, Inc., Reston, VA) using illuminant A and 10° observer (incandescent light) with a 0.25 in. port insert. Samples were overwrapped with clear saran film and surface color was measured at two locations (center and end) of each frankfurter. Five links from each treatment were measured for color. For texture measurement, a Texture Analyzer (Model TA-XT 2 i, Godalming, U.K.) was used for assessment of skin toughness and interior firmness by measuring surface puncture resistance and interior textural firmness with a 3-mm puncture probe. Five frankfurters from each treatment were measured at the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm/s. All samples were measured at room temperature 3 h after removal from the refrigerator.

For pH measurement, 10 g of sample was blended with 90 ml distilled water in a Waring Blender® and the slurry measured with a pH meter (Fisher Accumet Model 925, Fisher Scientific Pittsburgh, PA) using a sealed combination electrode (Omega Engineering, Inc., Stamford, CT). The TBA values were measured using the modified method for cured meats (Zipser and Watts, 1962). Duplicate measurements of pH and TBA values were recorded for each sample.

**Sensory evaluation**

The sensory evaluation was conducted using a panel of 16 trained panelists, all being students, staff or faculty in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were
volunteers and were trained using commercial frankfurters, for the odor notes and terminology to be measured, and the scales used. Only uninoculated samples were used for sensory evaluations. Panelists evaluated samples for purge, color, texture and odor using a 15-cm unstructured line scale. Fluorescent-lighted booths were used for sample presentation to the panelists.

The amount of purge and exterior color was evaluated using intact unopened packages of frankfurters. For the texture assessment, the panelists used the edge of a dinner fork to cut through the center of a frankfurter section. For odor evaluation, frankfurters were heated in boiling water for 2 min, cut into sections and placed into 150-ml covered containers before presentation to the panel. Panelists evaluated odor of the frankfurters for smoky, burnt and acidic traits; all of which were established during the training sections. The numerical scales used for sensory intensity of purge, color, texture and odor, were described as; 0 = none, extremely light, extremely soft or none, respectively; while 15 = extremely abundant, dark, firm or intense, respectively. All sensory evaluations were conducted within two weeks of processing and were repeated three times.

**Comparison of delivery systems for pediocin**

Two alternative systems for delivery of pediocin and other inhibitors were evaluated for their inhibitory effectiveness and compared to the spraying method used in the previous experiments. The first method consisted of a commercial cellulose casing (XTRA™-Protect, Alfacel, Inc., Woodridge, IL) which has an interior coating of either pediocin or pediocin plus sodium diacetate. These casings were used for frankfurter manufacturing as previously described. After peeling, the frankfurters were packaged and inoculated with 3.40 log CFU/g of the 5-strain mixture of *L. monocytogenes*. The second system for delivery of pediocin was a co-extrusion of collagen dough containing a 40% ALT A™ 2341 suspension. The collagen was co-extruded with the frankfurter emulsion batter (Townsend Kontura, Townsend Engineering, Des Monies, IA) to form a casing over the links as they were extruded. Frankfurters with the exterior co-extrusion casing including pediocin were then packaged and surface-inoculated as described previously. Frankfurters sprayed with pediocin were used to compare the immediate inhibitory effectiveness of these two delivery systems with the surface-spray application.

**Statistical analyses**

Microbiological data were transformed into logarithms of the number of colony-forming units (log_10 CFU/g). The Statistical Analysis System (SAS, 2001) was used to determine means, standard errors and variance analyses from three replications. When analysis of variance (ANOVA) revealed a significant difference (P < 0.05), treatment means were compared using the least significant difference (LSD) test.

**Initial evaluation of treatments.** Data from the initial evaluation were treated as a split-plot design with *L. monocytogenes* inoculation level and package size as the main plot, and pediocin concentration as the split plot. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey’s range test for least significance differences.

**Evaluations during storage.** The data from the evaluations during storage was analyzed as a split plot with 3 treatments in the main plot while the subplot consisted of 11-13 sampling dates. The sampling dates
were either day 0 to day 10 or week 0 to week 12 depending on storage temperatures. All data were analyzed using the general linear model (GLM) procedure. Comparisons of means were based on Tukey's range test for least significant differences.

Results and Discussion

Recovery of *L. monocytogenes*. *L. monocytogenes* was not detected on uninoculated frankfurters using MOX plates and very few aerobic bacterial counts (A.P.C.) were detected on TSA plates. After inoculation of frankfurters with *L. monocytogenes*, 3.40 or 5.20 log CFU/g of a 5-strain mixture of the pathogen was recovered from the original inoculations of 7.30 and 9.30 log CFU/ml (data not shown), respectively.

The results from this study confirm that *L. monocytogenes* survives well on frankfurters after inoculation. These results support Wang and Muriana (1994) who reported that different brands of retail frankfurters tested positive for *L. monocytogenes*. In that study, the pathogen was isolated from a variety of frankfurters types including chicken, beef/pork/turkey, beef and chicken/pork types. The results from several other studies have also indicated that retail wieners or frankfurters may support growth of *L. monocytogenes* (Glass and Doyle, 1989; Buncic et al., 1991; Mckellar et al., 1994; Wang and Muriana, 1994). This situation presents a potential hazard in consuming uncooked frankfurters or in cross-contamination of other foods by handling exudate from frankfurter packages.

The recoveries of the inoculated pathogen were less than inoculated levels probably due to nonmeat ingredients and composition of the frankfurters. *L. monocytogenes* recovery has been reported to be improved by using the United States Department of Agriculture - Agricultural Research Service (USDA-ARS) product composite rinse method and the USDA-ARS package rinse method (Luchansky et al., 2002). In general, the sampling methods in this study were very similar to the USDA-ARS methods. Environmental and processing conditions, such as storage temperatures, pH, atmosphere and particularly, food additives, can influence the survival and growth of *L. monocytogenes* (Doyle, 1988; Johnson et al., 1990). Glass and Doyle (1989) observed differences in the growth rate of *L. monocytogenes* on wieners from different manufacturers. Growth of *L. monocytogenes* may vary with the composition of meat products (Grau and Vanderlinde, 1992; Taormina and Beuchat, 2002), such as the content of fat and sodium. Growth is also influenced by nonmeat ingredients, such as residual nitrite (Grau and Vanderlinde, 1992; Schlyter et al., 1993b), polyphosphates (Giese, 1994) and phenols (Glass and Doyle, 1989; Glass et al., 2002). It is likely that phenols from smoke, phosphates, salt and nitrite contributed to less than 100% recovery of *L. monocytogenes* in this study.

Inhibitory effectiveness of pediocin (in ALTA™ 2341) on *L. monocytogenes*. The effects of pediocin at concentrations of 3,000 AU and 6,000 AU (Pdn-3000 and Pdn-6000) are shown in Fig. 1 for the three package types (1, 5 or 10 link[s] per package) and both inoculation levels. The results from MOX agar indicated that the pediocin had a significant (P < 0.001) inhibitory effect that was concentration-dependent. Regardless of package types, pediocin provided an immediate reduction of the pathogen populations by about 1.5 to 1.6 log CFU/g (Pdn-3000) or 1.8 to 2.1 log CFU/g (Pdn-6000) for inoculations of 3.40 or 5.20 log CFU/g
Figure 1. Survival of *Listeria monocytogenes* and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) at 3,000 AU or 6,000 AU; *A. L. monocytogenes* counts (3.40 and 5.20 log CFU/g inoculations) on MOX agar; B. Aerobic bacterial counts (A.P.C.) on TSA

**Pdn-3000**: Frankfurters (1, 5 & 10 links [s]) treated with 3,000 AU pediocin per link

**Pdn-6000**: Frankfurters (1, 5 & 10 links [s]) treated with 6,000 AU pediocin per link
L. monocytogenes, respectively (Fig. 1-A). The reduction of L. monocytogenes observed for the pediocin treatments was greater than the decrease of 0.9 log CFU/ml (4.5 log CFU/ml initial inoculation) reported by Schlyter et al. (1993b). The results of our study also indicated that the reduction of L. monocytogenes was increased with increased pediocin concentration. The pathogen number on high inoculum samples treated with Pdn-6000 was reduced more (P < 0.0001) than those treated with Pdn-3000 under the same conditions. Interestingly, the differences were not significant (P > 0.05) on the low inoculum samples (3.40 log CFU/g). Package types did not differ significantly (P > 0.05) from one another for antilisterial effects of pediocin.

The data (Fig. 1-B) from TSA without addition of yeast extract indicated that the high inoculation (5.20 log CFU/g) packages resulted in lower recovery than with MOX agar. However, the recovery from the low inoculation level (3.40 log CFU/g) was not affected by the type of agar used in this study. In general, the counts from TSA were higher than that from MOX agar. The TSA plates include other organisms in addition to L. monocytogenes though most of the counts from inoculated samples are likely to be L. monocytogenes. It is also likely that some of the cells of L. monocytogenes were injured rather than killed after treatment of pediocin. The injured cells can repair cellular damages better on TSA (nonselective agar) than on MOX agar (selective agar). Injured cells may recover and multiply in foods to become a potential risk (Bedie et al., 2001).

Because L. monocytogenes is a gram-positive bacteria, vitamin B complex is important to growth (Jay, 2002). Yeast extract provides vitamin B complex, and may further improve recovery of injured L. monocytogenes. Consequently, for the remainder of the study involving storage; TSA plus 0.6% yeast extract (TSA-YE) was used to maximize potential recovery of L. monocytogenes.

According to previous investigations and the current results, it is clear that pediocin has potential to reduce or inhibit the growth of L. monocytogenes (Pucci et al., 1988; Bhunia et al., 1991; Bruno and Montville, 1993; Chen and Montville, 1995). Pediocin has been studied in a variety of meats (Nielsen et al., 1990; Ming et al., 1997; Murray and Richard, 1997), and processed meat products, including frankfurters (Berry et al., 1991), wiener (Degnan et al., 1992) and wiener exudates (Yousef et al., 1991). The bactericidal action of pediocin AcH against Listeria spp. has been reported to be unaffected by the meat used (Motlagh et al., 1992).

Nielsen et al. (1990) reported that beef samples inoculated with L. monocytogenes and then treated with pediocin reduced counts by 1.0 to 2.5 logs compared with control samples. The reduction of pathogen numbers increased with increasing concentrations of pediocin, similar to our finding and the results of Motlagh et al. (1992). Greater antilisterial effectiveness was reported when population levels of the pathogen were lower (Nielsen et al., 1990; Motlagh et al., 1992), but this was not observed in our study. This may be due to the volume of pediocin solution or L. monocytogenes inoculum used in our study.

The reduction in numbers of the pathogen was greater when a smaller inoculum (10⁴ CFU/ml) was used regardless of whether the meat was treated with pediocin before or after exposure to L. monocytogenes (Nielsen et al., 1990). However, the data indicated that treatment of beef with pediocin before exposure to L. monocytogenes was more effective than application after contamination (Nielsen et al., 1990). In our study,
frankfurters were treated with pediocin before exposure to *L. monocytogenes*. The inhibitory effectiveness of pediocin on frankfurters already contaminated with *L. monocytogenes* should also be considered.

**Survival and growth of *L. monocytogenes* on frankfurters during storage at different temperatures**

**Storage at 4°C.** The growth of *L. monocytogenes* in the presence of pediocin at 4°C is shown in Fig. 2. Pediocin treatments significantly (P < 0.05) reduced the *L. monocytogenes* counts (MOX) compared to those on untreated control samples (Fig. 2-A). No growth occurred for 7 weeks after which the population appeared to increase, but did not exceed 4.02 log CFU/g throughout storage for 12 weeks. The results for control samples are similar to the study by Porto et al. (2002), where *L. monocytogenes* numbers in untreated control samples increased to 5.0 log CFU per package from an initial inoculation level of 500 CFU per package. Samelis et al. (2002) reported that pathogen numbers on frankfurters, stored at 4°C, reached 6 log CFU/cm² after 20 days and increased further to a level of 8 log CFU/cm² after 50 days following an initial inoculation level of 3.9 log CFU/cm². The higher final counts reported by Samelis et al. (2002) may be due to inoculation mixtures and strains used. Both our study and Porto et al. (2002) used a 5-strain mixture of *L. monocytogenes*, but the strains were different. Samelis et al. (2002) used a 10-strain mixture of *L. monocytogenes*.

Both treatments at 4°C with pediocin, at either level, delayed growth of *L. monocytogenes* for 7 weeks (≤ 1.99 log CFU/g). While the growth, in Fig. 2, appears to increase after 7 weeks, there were no significant (P > 0.05) differences among the different sampling times. Throughout the storage time, the numbers for *L. monocytogenes* from pediocin-treated groups were lower than the control group and this difference was significant (P < 0.05) for the first 7 weeks. After storage for 7 weeks, there was no significant (P > 0.05) difference between pediocin-treated samples and control samples. Nielsen et al. (1990) reported similar results. The aerobic bacterial populations counted by aerobic plate counts (A.P.C.), using TSA-YE (Fig. 2-B) showed similar patterns to those using MOX agar (Fig. 2-A). Therefore, pediocin treatments can inhibit the growth of *L. monocytogenes* on frankfurters stored at 4°C. Our results also confirm that *L. monocytogenes* can survive well at refrigeration temperatures and refrigeration alone is relatively ineffective for inhibiting the growth of *L. monocytogenes*. These results are similar to reports from Buncic et al. (1991) and Glass and Doyle (1989), who found that *L. monocytogenes* can survive and grow at 4.4°C on the surface of vacuum-packaged frankfurters, ham, bologna, sliced chicken and turkey products, wieners and fresh bratwurst.

**Storage at 10°C.** In Fig. 3, the results of storage at 10°C are shown for the control and two pediocin treatments. As at 4°C, there was no difference in effects between the two pediocin concentrations. Growth of surviving cells of *L. monocytogenes* (Fig. 3-A) was more rapid on pediocin-treated samples stored at 10°C for up to 12 weeks (≤ 6.29 log CFU/g), than on those stored at 4°C. However, growth of the pathogen was delayed about 2 weeks at 10°C, with counts of 2.80 to 3.03 log CFU/g, as opposed to about 7 weeks at 4°C. After 3.5 weeks, the pathogen numbers in the pediocin-treated samples reached 5.07 to 5.24 log CFU/g, similar to those of the control, and remained similar for the rest of the storage period. Overall, samples treated with pediocin were not significantly (P > 0.05) different from the control group throughout storage for 12 weeks. However, for the samples stored during the first 2 weeks the pediocin treatments were significantly (P < 0.05) lower than
A. *L. monocytogenes* (MOX)

B. Aerobic bacteria (TSA-YE)

Figure 2. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) at 3,000 AU or 6,000 AU and stored at 4°C; A. *L. monocytogenes* on MOX agar, B. Aerobic bacterial counts (A.P.C.) on TSA-YE

Pdn-3000- Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
Pdn-6000- Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
B. Aerobic bacteria (TSA-YE)

Figure 3. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) at 3,000 AU or 6,000 AU and stored at 10°C; A. *L. monocytogenes* on MOX agar, B. Aerobic bacterial counts (A.P.C.) on TSA-YE.

Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
controls.

The A.P.C. on TSA-YE (Fig. 3-B) had higher numbers than on MOX agar (Fig. 3-A). The differences between these 2 agars were less than that observed at 4°C. This may suggest that repair system(s) or enzymes, which are greatly influenced by temperatures, may function better for recovery at 10°C than 4°C. Pediocin did not inhibit the growth of *L. monocytogenes* on frankfurters as well at 10°C as at 4°C. The numbers of surviving cells observed on MOX and on TSA-YE were very similar at any given storage time.

**Storage at 25°C.** When samples were held at 25°C (Fig. 4), only a slight delay in growth of pediocin-treated *L. monocytogenes*, at counts of 2.47 to 2.81 log CFU/g, occurred during the first day. After storage for 4 days, the pathogen counts (Fig. 4-A) reached 4.79 to 5.38 log CFU/g, similar to those of control samples, and remained similar to the controls for the remainder of the storage period. Growth of *L. monocytogenes* was much more rapid on frankfurters stored at 25°C than at 4°C and 10°C. There was no significant (P > 0.05) difference in counts between pediocin-treated samples and control samples throughout storage for 10 days, but pediocin-treated samples appeared to delay growth for 1 day. Again, as at 4°C and 10°C, there were no differences between the two concentrations of pediocin. Results at 25°C were similar to those observed at 10°C except for the shorter lag phase for *L. monocytogenes* growth.

The A.P.C. numbers on TSA-YE (Fig. 4-B) again was similar though higher (P > 0.05) than those from MOX agar. Thus, even at 25°C, pediocin will suppress initial growth of *L. monocytogenes* for a short period.

**Storage temperature effects.** While the initial reduction in pathogen numbers depended on the addition of pediocin, the length of the subsequent lag phase was dependent on the storage temperatures. Refrigeration temperatures delayed the growth of *L. monocytogenes*. The results of this study showed that this pathogen can survive and grow on frankfurters over a broad ranges of temperatures, from 4°C (likely storage temperature of retail display cases in supermarkets), to 10°C (potential storage temperature of home refrigerators) or 25°C (abuse temperature). There was no significant concentration effect of the pediocin as used in this study at any of the storage temperatures. The results confirmed that storage temperature is an important factor in control of *L. monocytogenes* growth but cannot inhibit the organism without additional barriers. Package types did not affect the inhibitory effects of pediocin in this study (P > 0.05).

Glass and Doyle (1989) emphasized the importance of preventing post-processing contamination of RTE meat products with *L. monocytogenes*. The long-held expectation that refrigerated storage at 4°C to 7°C will prevent the growth of foodborne pathogens clearly is not valid for *L. monocytogenes*. These authors pointed out that the refrigerated temperatures (0°C to 10°C) will not prevent growth of *L. monocytogenes*; but will extend the time before growth occurs and will reduce the rate of growth. These effects become greater with lower storage temperatures (Walker et al., 1990). Because meat manufacturers can no longer completely rely on refrigerated storage for pathogen control, Glass and Doyle (1989) recommended novel, non-traditional measures, such as antimicrobial agents, reduced temperature (< 2°C) storage, reformulation of products, or post-processing pasteurization of products should be included for the control of *L. monocytogenes* in meats.
Figure 4. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) at 3,000 AU or 6,000 AU and stored at 25°C; A. *L. monocytogenes* on MOX agar, B. Aerobic bacterial counts (A.P.C.) on TSA-YE

Pdn-3000- Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
Pdn-6000- Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
By itself, pediocin will not control the growth of surviving cells (Motlagh et al., 1992). Motlagh et al. (1992) reported that surviving cells of L. monocytogenes in pediocin AcH-treated food samples multiplied in the presence of residual pediocin AcH during storage at 4°C and 10°C. Following an initial decrease in numbers at 25°C, the results for pediocin alone were similar (4 log CFU/ml increase within 7 days) to the control (Schlyter et al., 1993b). These results suggest that pediocin AcH is not bacteriostatic to Listeria cells. However, the results from our study indicated that pediocin had a bacteriostatic effect on Listeria cells regardless of storage temperatures. The pediocin concentrations used in our study are 3,000 AU or greater, at least twice the concentrations used by Motlagh et al. (1992), which may explain the greater effectiveness. Lower refrigeration temperatures slowed the growth of L. monocytogenes on frankfurters treated with pediocin. The data from our study showed that pediocin-treated samples stored at 4°C had an initial listericidal effect (day 0) and was bacteriostatic for survivors during the first 7 weeks of storage.

Pediocin delayed the growth of L. monocytogenes on frankfurters stored at 10°C, but it was more effective when samples were stored at 4°C. Glass et al. (2002) also reported that a lower temperature enhanced the antilisterial activity of organic acids on unsmoked bratwurst. Consequently, manufacturers can utilize these combined treatments to help protect frankfurters or other RTE processed meats from L. monocytogenes. If frankfurters are stored in a home refrigerator (around 10°C), storage probably should be no longer than 2 weeks. These results indicate that while pediocin is effective, the significance of maintaining the proper temperature of refrigerated foods such as frankfurters is also very important.

**Product quality analyses**

Addition of pediocin to frankfurters was evaluated for potential product quality changes and the results for purge, color, texture, odor, pH and oxidative change (TBA values) are presented in Table 1. There was no effect of treatments on packaging purge except for the volume of solution added to packages. The addition of pediocin, for example, as 1ml or 2 ml of solution, resulted in a significant difference in the water measured as purge. It is clear that the purge accumulation of frankfurters in packages with pediocin added, at either level, is significantly different from that of control samples. Purge was significantly affected by the type of package as might be expected with the greater number of frankfurters per package resulting in greater purge (data not shown).

Addition of pediocin resulted in color which was darker (L* value) and redder (a* value). These differences are not large but were statistically significant (P < 0.05) at the high concentration of pediocin. The pH values were lower (P < 0.05) with addition of pediocin than that of controls. Textural characteristics were not affected by any of the product treatments, either for exterior skin toughness or for interior firmness. TBA values of products were also unaffected by the treatments.

**Sensory evaluation**

Sensory panelists evaluated frankfurters for visual purge, visual external color, texture and specific preselected aroma. The results are shown in Table 2. The purge assessments reflect the volume of added solutions such as the 1 ml (3,000 AU) or 2 ml (6,000 AU) of pediocin. The control was significantly
Table 1. Comparison of physical and chemical analyses of frankfurters following treatments with pediocin (in ALTA™ 2341)

<table>
<thead>
<tr>
<th></th>
<th>% Purge</th>
<th>Color</th>
<th>Firmness (kg)</th>
<th>pH</th>
<th>TBA (mg MDA/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
<td>Skin</td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ± 0.25</td>
<td>44.98 ± 0.90</td>
<td>15.14 ± 1.12</td>
<td>13.67 ± 0.90</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>Pdn-3000¹</td>
<td>3.33 ± 0.62</td>
<td>44.38 ± 1.50</td>
<td>15.76 ± 0.97</td>
<td>13.35 ± 0.71</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td>(ab)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>Pdn-6000²</td>
<td>5.13 ± 0.67</td>
<td>42.93 ± 1.03</td>
<td>15.93 ± 1.41</td>
<td>13.19 ± 0.49</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>(c)</td>
<td></td>
<td>(b)</td>
<td>(a)</td>
<td>(b)</td>
<td>(a)</td>
</tr>
</tbody>
</table>

1. Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU
2. Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU

Means in the same column with different letters are significantly (P < 0.05) different
Table 2. Sensory evaluation\(^1\) of frankfurters following treatments with pediocin (in ALTA™ 2341)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge</th>
<th>Color</th>
<th>Texture</th>
<th>Aroma</th>
<th>Aroma</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Smoky</td>
<td>Burnt</td>
<td>Acidic</td>
</tr>
<tr>
<td>Control</td>
<td>1.29 ± 0.17</td>
<td>5.98 ± 0.55</td>
<td>7.10 ± 0.95</td>
<td>7.04 ± 0.50</td>
<td>1.60 ± 0.36</td>
<td>4.63 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>Pdn-3000(^2)</td>
<td>5.41 ± 1.73</td>
<td>6.99 ± 1.67</td>
<td>8.12 ± 1.03</td>
<td>7.46 ± 0.41</td>
<td>1.98 ± 0.37</td>
<td>4.75 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>(b)</td>
<td>(a)</td>
<td>(ab)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>Pdn-6000(^3)</td>
<td>9.19 ± 0.39</td>
<td>8.10 ± 1.42</td>
<td>9.50 ± 1.14</td>
<td>7.41 ± 0.53</td>
<td>2.16 ± 0.30</td>
<td>4.35 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>(a)</td>
<td>(b)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
</tbody>
</table>

1. The numerical scales of sensory intensity use for purge, color, texture, aroma of smoky, burnt and acid were: 0 = none, extremely light, extremely soft, and none, respectively; and 15 = extremely abundant, dark, firm, and intense, respectively.

2. Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
3. Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin

Means in the same column with different letters \(^{a-ca}\) are significantly (P < 0.05) different.
lower for apparent purge than the treatments because there were no added solutions. The trend of color scores of the frankfurters (Table 2) was lower for the control samples compared with the 2 treatments but this was not significant.

Odor scores of the samples, evaluated as smoky, burnt and acidic aromas, did not differ and the experimental treatments did not result in significant changes in product odors. The texture assessment by the sensory panel indicated the addition of pediocin increased the product resistance to slicing at the higher level (Pdn-6000) of pediocin addition. However, the scores (9.50/15.0 points) were still located in intermediate area of the scale used by the panelists. Instrument evaluations of texture showed no significant (P > 0.05) differences among the treatments, or between the treatments and the control for either skin toughness or interior firmness of the frankfurters (Table 1).

Glass et al. (2002) suggested that surface application of antimicrobials had less impact on the sensory and functional properties of the product than inclusion in the product formulation. Surface treatment of the cooked meat products with antimicrobial solutions can serve an intervention treatment and will deliver the antimicrobial agents at the point of contamination after chilling and before packaging.

**Comparison for inhibitory effects of pediocin by different means of delivery**

Two different systems, each of which might serve to deliver pediocin and other inhibitors to the surface of frankfurters, were evaluated in addition to the spray application used for this study. For the first, commercial cellulose casings with an interior coating of either pediocin or pediocin plus sodium diacetate were used for frankfurter manufacturing. This treatment resulted in a small reduction of *L. monocytogenes* compared to the control, but the difference was not significant (Table 3). This is, however, an extremely easy system to use and, if effectiveness can be increased, it would offer an easy delivery system. Indirect approaches used to apply bacteriocin or antimicrobials to food packaging materials have been reported to have potential for effective control of *L. monocytogenes* (Ming et al., 1997). The application of antimicrobials on the internal surface of films with resulting inactivation of *L. monocytogenes* on meat products has been reported (Ming et al., 1997).

The second system evaluated for delivery of the pediocin was a co-extrusion of collagen dough with the frankfurter emulsion/batter to form an exterior casing over the meat mixture. Pediocin was incorporated into the collagen dough prior to co-extrusion. Results from co-extruded, inoculated frankfurters showed a small, but insignificant reduction of *L. monocytogenes* numbers, similar to results with the cellulose casings lined with pediocin. The co-extrusion process, however, offers potential for manipulating the amounts of pediocin or other compounds added and has potential for further investigations.

**Conclusions**

Results of this study showed that pediocin activity in the commercially available ALTA™ 2341 significantly reduced the number of *L. monocytogenes* on packaged frankfurters and delayed growth of the remaining cells during storage. The extent of time delay was highly temperature-dependent. Storage at 4°C
Table 3. Comparison of different delivery systems for inhibitors of *Listeria monocytogenes* (3.40 log CFU/g inoculation) on the surface of frankfurters

<table>
<thead>
<tr>
<th>Agars 1</th>
<th>Normal Casing</th>
<th>Special Casing 2</th>
<th>Co-extrusion 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Pdn-6000 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pdn</td>
<td>Pdn + S.D.</td>
<td></td>
</tr>
<tr>
<td>MOX</td>
<td>3.53 ± 0.08</td>
<td>3.02 ± 0.09</td>
<td>3.61 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(ac)</td>
<td>(b)</td>
<td>(a)</td>
</tr>
<tr>
<td>TSA - YE</td>
<td>3.65 ± 0.05</td>
<td>3.15 ± 0.09</td>
<td>3.58 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(a)</td>
<td>(b)</td>
<td>(a)</td>
</tr>
</tbody>
</table>

Means in the same column with different letters 4-5 are significantly (P < 0.05) different

1. Agars for *L. monocytogenes* recovery
2. Interior casing coating of pediocin or pediocin with sodium diacetate (S.D.)
3. Townsend Kontura system
4. Pdn-6000: Frankfurters (5 links/pkg) sprayed with 6,000 AU pediocin
5. Pediocin-included
slowed the growth of *L. monocytogenes* on frankfurters treated with pediocin. Frankfurters treated with 6,000 AU of pediocin and stored at 4°C inhibited the growth of *L. monocytogenes* for up to 12 weeks. Therefore, pediocin provides an effective intervention treatment for *L. monocytogenes* on frankfurters. However, a single intervention is generally insufficient for *L. monocytogenes* control if temperature is not well controlled. The safety of RTE meats can be enhanced when antimicrobials such as pediocin are used in combination with diligent HACCP programs and environmental control programs for *L. monocytogenes*.

Most of the quality characteristics of the frankfurters measured in this study were unaffected by the pediocin treatments. The exceptions included color changes in lightness and redness. Products with pediocin were darker and redder, but the differences relative to controls were small. Use of pediocin is unlikely to result in quality changes that would be perceived as different by consumers.

While dipping or spraying are effective methods for surface treatment of frankfurters with antimicrobials, other methods such as coated casings and co-extrusion of casings are much easier to use. Further work with these systems to increase the microbial impact of the antimicrobials by these delivery systems is recommended.

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CHAPTER 4. EFFECTS OF PEDIOCIN AND POST-PACKAGING THERMAL PASTEURIZATION ON LISTERIA MONOCYTOGENES ON FRANKFURTERS

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Abstract

Frankfurters, in different package types (single link, 5 links and 10 links per package), were surface-inoculated with a 5-strain mixture of Listeria monocytogenes (3.40 or 5.20 log CFU/g) after treatments with 3,000AU or 6,000 AU pediocin (in ALTA™ 2341) per link (Pdn-3000 or Pdn-6000). The frankfurters were vacuum-packaged, after which the packages were heated in hot water at 71°C, 81°C or 96°C for 30, 60 or 120 s. L. monocytogenes was enumerated following the treatments. Selected treatments were subsequently evaluated during storage at 4°C, 10°C and 25°C for up to 12 weeks.

L. monocytogenes was reduced by all treatments (single-link » 5-link > 10-link packages), but 81°C or more for at least 60 s with pediocin (Pdn-6000) was necessary to achieve a 50% reduction of initial inoculations. Storage at 4°C effectively slowed the growth of this pathogen in all package types. Little or no growth of L. monocytogenes occurred on frankfurters in single-link packages for 12 weeks at 4°C or 10°C, and for 12 days at 25°C. Thus, temperature alone had a significant effect on L. monocytogenes. The smaller packages (< 5 links per package) allowed greater antilisterial effects of PPTP treatments than 10-link packages. Single-link packages generally resulted in the greatest reduction of L. monocytogenes counts following PPTP treatments. Generally, the treatments mentioned above did not significantly (P > 0.05) affect the sensory qualities of frankfurters.

Introduction

Post-processing contamination of ready-to-eat (RTE) processed meat products by L. monocytogenes, represents a serious health risk (10,11) and has become a major concern for the meat processing industry (47). L. monocytogenes is widespread and difficult to avoid before or during packaging (3). Additional intervention hurdles are required in the form of pre- or post-packaging technologies to control the growth of this pathogen and to enhance the safety of these products during storage (3).

Samelis et al. (49) emphasized that L. monocytogenes can be resistant to many food preservation approaches (32), colonize in meat plants (48) and survive under unfavorable conditions (22, 48). L. monocytogenes was often isolated from floors, drains, cleaning aids, walls, ceilings and critical control point (CCP) areas in food processing facilities (43, 48,52, 53).
Unfortunately, sanitation strategies and hygienic practices applied in plants are often insufficient to prevent recontamination and growth of *L. monocytogenes* on processed meat products (48, 53). Extensive efforts to control *L. monocytogenes* can reduce the level of contamination, but eradication from the processing environments or from all finished products is not considered possible (4). Several studies have reported that meat products testing positive for *L. monocytogenes* at retail stores harbored the pathogen typically at levels of $10^3$ CFU/g or less (19, 23). Thus, post-packaging hurdle technologies that will reduce *L. monocytogenes* numbers by similar amounts are needed to control *L. monocytogenes* in meat products during storage (4).

Problems of *L. monocytogenes* contamination typically develop from post-thermal process contamination because the pathogen is relatively heat-susceptible and is normally killed by typical thermal processes used for RTE processed meats. Thus, it is practically feasible to inactivate this pathogen by the type of mild heat treatment given to minimally processed foods without negatively impacting the product quality. Samelis et al. (49) suggested that post-packaging thermal pasteurization (PPTP) (45) also may increase the antilisterial effects of antimicrobials, such as pediocin, in cooked cured meats. Because initial levels of *L. monocytogenes* are typically low, a 1- to 2-log reduction in surface contaminants of commercially manufactured products may be adequate to completely inactivate or reduce the pathogen populations to undetectable levels. At low population levels, heat-stressed cells are less likely to recover and thus become more susceptible to antimicrobial treatments (47). Roering et al. (45) concluded that thermal pasteurization, at temperatures of 77°C or greater for at least 60 s, was sufficient to eliminate appreciable numbers of *L. monocytogenes*, regardless of other factors. Assuming no further handling or processing of the products after packages are sealed, the pasteurization temperatures and times reported should be sufficient to eliminate relatively low levels of the pathogen. *L. monocytogenes* is also relatively more heat-resistant compared to some other pathogens, such as *Salmonella* spp., and *Escherichia. coli* O157: H7 (17, 25, 33). Thus, PPTP treatments may also have extra benefits; for example, reducing levels of *E. coli* O157: H7 or *Salmonella* spp., on processed meat products (45).

Pediocin has been studied in a variety of meat applications (38, 41, 42), including frankfurters (5) and wiener (16) to reduce or inhibit the growth of *L. monocytogenes* (12, 44). Spraying or dipping of products in antimicrobial (pediocin) solutions before packaging (49) combined with PPTP treatments (45) could enhance the effectiveness of both antimicrobial treatments. The results from a study by Samelis et al. (47) indicated a limited effectiveness of PPTP treatments against *L. monocytogenes* in the absence of antimicrobials and this may have significant practical implications. Manufacturers should not rely solely on one method without previous evaluation of its effectiveness for different products.

Post-processing application of antimicrobials directly on the product surface where *L. monocytogenes* cells may be present is more advantageous than addition to the formulation (53). In addition to the potential benefit of providing increased antilisterial effects, combinations of antimicrobials or treatments may lessen any negative effects on the sensory quality of cured meat products (47) when applied to the surface as opposed to formulation as part of the product.
The objective of this study was to assess the inhibitory effectiveness of pediocin (in ALTA™ 2341) in combination with PPTP treatments on L. monocytogenes on the surface of frankfurters in different package types (1-link, 5-links and 10-links per package). Subsequently, samples treated with selected antimicrobial treatments were stored at 4°C, 10°C and 25°C for up to 12 weeks. Samples were collected periodically and analyzed for microbial growth. Moreover, the physical and chemical properties, including sensory quality, of frankfurters, subjected to selected antimicrobial treatments, were measured.

Materials and Methods

The experimental protocol was designed to first compare the effects of pediocin concentration and PPTP treatment on inhibition of L. monocytogenes on frankfurters in different package types. The most effective combinations were then evaluated for inhibitory effects during storage at 4°C, 10°C and 25°C. Finally, the most effective treatments were assessed for product quality changes.

Frankfurter preparation

Frankfurters were manufactured from frozen beef trim (~80% lean) and fresh pork trim (~50% lean) purchased from commercial suppliers. Frozen beef was removed from the freezer (-20°C) 24 h before processing and tempered at 2°C to 4°C. All trimmings were coarse ground (0.95-cm plate) and fat content measured by Anyl-Ray (Kartridg-Pak Co., Davenport, IA). Frankfurter batches were formulated using a 45.4 kg meat block, including 22.7 kg of beef trim and 22.7 kg of pork trim, plus 9.1 kg of ice/water, 1.49 kg of spice mixture (A.C. Legg Packing Co., Inc., Birmingham, AL), 112 g of curing salt (A.C. Legg Packing Co., Inc., Birmingham, AL) and 908 g of salt.

The lean beef trim was chopped first with ice/water, salt, spices and cure to about 4.5°C after which the fat pork trim was added and chopping continued to 13.9°C to form the batter. The frankfurter batter was vacuum-stuffed (Risco Model RS 4003-165; Stoughton, MA) into 24 mm cellulose casings and linked at 12 to 14 cm in length. Stuffed frankfurters were smoked, cooked and showered in an Alkar oven (Alkar, Lodi, WI). Chilling was achieved at 2°C overnight before peeling. Finished, peeled frankfurters were stored at 2°C for 12 to 18 h before random assignment to different experimental treatments. Finished weight of frankfurters was 45 ± 5 g per link.

Fat, moisture (1) and protein contents (2) of finished products were measured. The results indicated the frankfurters contained 28.25% crude fat, 53.66% moisture and 13.44% protein.

Inoculation and packaging

Frankfurters were packaged in vacuum bags using three different package types as follows: 1) 10 links per package in a double row of 5 each, 2) 5 links per package in a single row, and 3) singly packaged links. Each packaging group was divided into two subgroups for treatment with one of two levels of pediocin (ALTA™ 2341, Quest Int., Sarasota, FL) before inoculation with L monocytogenes. ALTA™ 2341 was formulated at 40% (wt/vol) in sterile, distilled water to achieve 3,000 arbitrary units (AU) of pediocin per ml.
The frankfurters were placed in bags, then sprayed with either 1 or 2 ml per link of 40% ALTA™ 2341 suspension, equivalent to 3,000 AU or 6,000 AU pediocin per link (Pdn-3000 or Pdn-6000). The vacuum bags (Cryovac® B-2540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100°F, 100% RH/at 100 sq. in/24 h; oxygen transmission rate = 3-6 CC at 40°F/m²/24 h/0 % RH) with frankfurters and pediocin were hand-massaged for 5 s to 10 s for even distribution of pediocin, before vacuum-sealing (Multivac A 300/52, Mutivac Sepp Haggenmuller GmbH & Co., Wolfertschwenden, Germany).

For inoculation of the frankfurters, a 5-strain cocktail mixture of *L. monocytogenes* cultures, including *L. monocytogenes* Scott A, H7764 1/2a, H7969 4b, H7962 4b and H7762 4b was used. With exception of the Scott A strain, all strains were obtained as clinical isolates from the Bil Mar Foods outbreak of 1998-1999 (CDC, Atlanta, GA). The cultures were individually grown in trypticase soy broth plus 0.6% yeast extract (TSB-YE broth) (Difco, Becton Dickinson and Company, Sparks, MD) at 35°C for 24 h. Then, 1 ml of culture from each individual strain was combined to give 5-ml mixed culture of *L. monocytogenes*. The mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35°C for 24 h to reach the stationary phase. The final concentration of the original 5-strain mixture of *L. monocytogenes* was 9.30 log CFU/ml.

Inoculation of frankfurters was done by adding 1 ml per link of the original 5-strain mixture of *L. monocytogenes* (9.30 log CFU/ml) or of a 1:100 (7.30 log CFU/ml) dilution of the original 5-strain mixture to result in a “high” or “low” inoculation level. The inoculum was added to the frankfurters after they were placed in the appropriate package and before the package was sealed. Inoculated frankfurters without addition of pediocin served as control samples and were used to determine the recovery of *L. monocytogenes*.

After inoculation, the packages were hand-massaged 5-10 s to evenly distribute the 5-strain mixture of *L. monocytogenes* on the surface of each frankfurter, and closed under vacuum. After vacuum-packaging, all frankfurters were placed in cardboard boxes and stored at 2°C to 4°C for 14-18 h before sampling. Averaged recovery of *L. monocytogenes* from untreated samples showed inoculated products to have 3.40 or 5.20 log CFU/g, respectively for the two inoculation levels.

**PPTP treatments**

For PPTP treatments, samples were inoculated as described prior to PPTP treatments. Pasteurization temperatures included 71°C (± 1°C), 81°C (± 1°C), or 96°C (± 2°C), each for 30, 60 or 120 s. A water bath (Fisher Isotemp-220, Fisher Scientific, Pittsburgh, PA) and Fisher immersion circulator (Model 730, Fisher Scientific, Pittsburgh, PA) were used to control water temperature. Packages were held in heated water for the prescribed time, then immersed in 15°C water for 5-10 min to chill before placement in refrigerated storage at 2°C-4°C.

**Initial evaluation of treatments**

Survival of the inoculated *L. monocytogenes* was determined by enumerating the surviving organisms. The inhibitory effectiveness of the treatment combinations was compared to determine which were the most effective combinations for subsequent study during refrigerated storage.
Evaluations during storage

Based on the initial evaluation of treatments, those treatments that showed the greatest immediate inhibitory effectiveness were selected for subsequent evaluation of long-term effectiveness during storage. Treatments that were selected included pediocin alone (Pdn-6000) at 6,000 AU, which provided greater reduction of *L. monocytogenes* than that of Pdn-3000, Pdn-6000 in combination with PPTP treatments at 81°C for 60 s (81°C/60 s), 96°C for 60 s (96°C/60s) or 96°C for 120 s (96°C/120s). The inoculation level used for storage evaluations was 3.40 log CFU/g of the same 5-strain mixture of *L. monocytogenes* used for the initial evaluation. Samples were stored at 4°C, 10°C and 25°C for up to 12 weeks. The untreated samples surface-inoculated with 3.40 log CFU/g served as control samples and were checked for recovery. The data from treatments with pediocin alone (13), was used to compare the antilisterial effects of the five selected treatments. All package types (single-link, 5-link and 10-link packages) were included in the storage time evaluation. The effect of the package type on the inhibitory effectiveness of *L. monocytogenes* was studied using the 96°C/120s treatment.

Microbiological analyses

**Initial evaluation of treatments.** Packages of frankfurters were aseptically opened, using sterile scissors, 18 h after treatments. Except for single-link packages, samples were collected by taking two frankfurters, one from the center of the package row and one from the outside. For 10-link packages, these two frankfurters were taken from different rows. Frankfurters were aseptically cut in half (ca. 20-23 g portions) with sterile scissors and tweezers, and homogenized (Seward Stomacher blender, Model 4000, Tekmar™ Co., Cincinnati, OH) at normal speed for two min in sterile stomacher bags (Whirl-Pak Filter Bag B01318, A Nasco, Ft. Atkinson, WI) with sufficient 0.1% sterile peptone water to give a 1:5 dilution of the sample. *L. monocytogenes* cells were enumerated by serially diluting 1 ml of the blended sample in 9 ml of 0.1% peptone water, plating on Modified Oxford (MOX) (Difco) agar plus 0.1% Oxford Antimicrobial Supplement. Samples were also plated on trypticase soy agar (TSA) (Difco). Incubation of all plates was at 35°C for 48 h. Typical *L. monocytogenes* colonies were enumerated, identified by gram stain and confirmed using API Listeria kits (bioMerieux, Inc., Hazelwood, MO).

**Evaluations during storage.** For enumeration of samples held in storage, packages were opened aseptically and whole frankfurters were mixed for 1 min in a Seward stomacher blender with an equal amount of 0.1% peptone water (50% dilution) to rinse them thoroughly. *L. monocytogenes* cells were enumerated by serial dilution suing 1 ml of the rinse in 9 ml of 0.1% peptone water, plating on MOX agar and TSA-YE (TSA plus 0.6% yeast extract) (Difco) with identification as described previously. When increased sensitivity was required, 1.0 ml samples of rinse were plated directly onto MOX or TSA-YE.

**Product quality analyses**

After completion of the microbiological evaluations, the treatments that had the most impact on *L. monocytogenes* were also evaluated for product quality changes. Frankfurters were prepared, processed and treated in the same way as for the inoculation challenge, but without the *L. monocytogenes* inoculation. All
frankfurters used for quality evaluations were packaged using 5 links per packages. Quality evaluations included measurement of purge, color, texture, odor quality, pH and thiobarbituric acid (TBA) values.

**Physical and chemical analyses of frankfurters**

For purge accumulation, two packages were each weighed, opened and the frankfurters removed. The packages and links were wiped dry and reweighed. The weight difference was calculated as purge and expressed as a percentage of unpackaged product weight (6).

Color ($L^*$, $a^*$, $b^*$) measurements utilized a Hunter Labscan spectrophotometer (Hunter Associated Laboratories, Inc., Reston, VA), using illuminant A and 10° observer (incandescent light) with a 0.25 in. port insert. Samples were overwrapped with clear saran film and surface color was measured at two locations (center and end) of each frankfurter. Five links from each treatment were measured for color. For texture measurement, a Texture Analyzer (Model TA.XT 2 i, Godalming, U.K.) was used for assessment of skin toughness and interior firmness by measuring puncture resistance and interior texture with a 3-mm puncture probe. Five frankfurters from each treatment were measured in the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm/second. All samples were measured at room temperature 3 h after removal from the refrigerator.

For pH measurement, 10 g of sample was blended with 90 ml of distilled water in a Waring Blender® and the slurry measured with a pH meter (Fisher Accumet Model 925, Fisher Scientific, Pittsburgh, PA) using a sealed combination electrode (Omega Engineering, Inc., Stamford, CT). The TBA values were measured using the modified method for cured meats (55). Duplicate measurements of pH and TBA values were recorded for each sample.

**Sensory evaluation**

The sensory evaluations were conducted using a panel of 16 trained panelists, all being students, staff or faculty in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were volunteers and were trained by using commercial frankfurters for the odor notes and terminology to be measured, and the scale used. Only uninoculated samples were used for sensory evaluation. Panelists evaluated samples for purge, color, texture, and odor using 15-cm unstructured line scale. Fluorescent-lighted booths were used for sample presentation to panelists.

The amount of purge and exterior color was scored using intact, unopened packages of frankfurters. For the texture assessment, the panelists used the edge of a fork to cut a cross-section thorough the center of a frankfurter. For odor evaluation, frankfurters were heated in boiling water for 2 min, cut into sections and placed in 150-ml covered containers before presentation to the panel. Panelists evaluated odor for smoky, burnt and acidic traits; all of which were established during the training session. The numerical scales used for sensory intensity of purge, color, texture and odor were described as; 0 = none, extremely light, extremely soft or none, respectively; while 15 = extremely abundant, dark, firm or intense, respectively. All sensory evaluations were conducted within two weeks of processing and were repeated three times.
Statistical analyses

Microbiological data were transformed into logarithms of the number of colony-forming units (log_{10} CFU/g). The Statistical Analysis System (46) was used to determine means, standard errors and variance analyses from the three replications. When analysis of variance (ANOVA) revealed a significant difference (P < 0.05), treatment means were compared using the least significant difference (LSD) test.

Initial evaluation of treatments. Data from the initial evaluation of treatments were treated as a split, split-plot design with *L. monocytogenes* inoculation level and packaging types as the main plot, pediocin concentration as the split plot, and combinations of pasteurization temperature and heating times as the split-split plot. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

Evaluations during storage. The data from the evaluations during storage was analyzed as a split plot with 8 treatments in the main plot while the subplot consisted of 11-13 sampling dates. The sampling dates were either day 0 to day 10 or week 0 to week 12 depending on storage temperature. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

Results

Initial evaluation of treatments

Inhibitory effects of pediocin (in ALTA™ 2341) and PPTP treatments on *L. monocytogenes*. No *L. monocytogenes* and very few aerobic bacteria were detected on uninoculated frankfurters. Survival of *L. monocytogenes* on inoculated frankfurters, treated with pediocin and PPTP, is shown in Fig. 1. For frankfurters with the lower initial inoculation level (3.40 log CFU/g), shown in Fig. 1-A, *L. monocytogenes* populations were reduced by 1.5 to 3.4 log CFU/g depending on the type of package. The inhibitory effect of pediocin was significant (P < 0.05), regardless of the other treatments. Treatment combinations (pediocin plus PPTP) resulted in greater reduction of *L. monocytogenes* counts than for pediocin-only treatments, regardless of package types (13).

Under the same conditions, frankfurters that were surface-inoculated with the higher initial level of *L. monocytogenes* (5.20 log CFU/g) showed greater reduction of *L. monocytogenes* (Fig. 1-C) than samples inoculated with 3.40 log CFU/g *L. monocytogenes* (Fig. 1-A). The reduction of *L. monocytogenes* was 1.9 to 5.2 log CFU/g for the high-inoculum pediocin/PPTP-treated samples (Fig. 1-C). The reduction achieved by the combined treatments was again greater than that for samples treated with pediocin only (13).

Inhibitory effects of PPTP on *L. monocytogenes*. The exposure of inoculated packages to hot water at 71°C, 81°C or 96°C for 30 s, 60 s or 120 s resulted in a wide range of inactivation effectiveness, regardless of other factors (Fig. 1). With an inoculation of 3.40 log CFU/g (Fig. 1-A), all treatments resulted in at least a 1.4-log reduction of *L. monocytogenes*. The single-link packages showed approximately a 3.0-log reduction except for the 30 s treatments at 71°C and 81°C. Twelve of the 18 treatment combinations shown in Fig. 1-A for
Figure 1. Survival of *Listeria monocytogenes* and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) and post-packaging thermal pasteurization (PPTP): A. *L. monocytogenes* counts on MOX agar (3.40 log CFU/g inoculation), B. Aerobic bacterial counts (A.P.C.) on TSA (3.40 log CFU/g inoculation), C. *L. monocytogenes* counts on MOX agar (5.20 log CFU/g inoculation), D. Aerobic bacterial counts (A.P.C.) on TSA (5.20 log CFU/g inoculation)

Pdn-3000: Frankfurters (1, 5 & 10 link[s] / pkg) treated with 3,000 AU pediocin per link
Pdn-6000: Frankfurters (1, 5 & 10 link[s] / pkg) treated with 6,000 AU pediocin per link
0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
single-link packages reduced *L. monocytogenes* to undetectable levels.

The 5-link packages showed a *L. monocytogenes* reduction of 1.5 to 3.0 logs with increased effectiveness when pasteurization temperature or heating time was increased. While the effects of increased pediocin concentration were significant (P < 0.05), the difference between 3,000 AU and 6,000 AU was not large. For 10-link packages, the population reduction of *L. monocytogenes* was 1.4 to 2.2 logs. There was less impact of increasing pasteurization temperature or heating time for packages with 10 links than observed for the other package types.

For packages inoculated with 5.20 log CFU/g (Fig. 1-C), results were similar to those observed for the 3.40 log CFU/g inoculation. All treatments resulted in at least a 1.9-log reduction of *L. monocytogenes*. The single-link packages showed nearly a 5.0-log reduction of *L. monocytogenes* at the higher temperature (81°C or greater), and the effects were more complete with the greater concentration of pediocin. Samples immersed in hot water at 96°C for at least 60 s or at 81°C for 120 s in combination with Pdn-6000 resulted in an undetectable level of *L. monocytogenes*. The 5-link packages showed ca. a 3.0-log reduction of *L. monocytogenes* at the most effective temperature (96°C). The effects of heating time were significant for 5-link packages but less marked than in the case of single-link packages. The effect of heating time and pediocin concentration was again significant (P < 0.05) but none of treatments achieved a complete (5.2 logs) reduction of *L. monocytogenes*. For 10-link packages, the population reduction of *L. monocytogenes* was 2.9 logs, at best. The effectiveness of increased pasteurization temperature and increased heating time was lessened by this package type. It is clear that the interfacial area between frankfurters that is not in contact with the package film protects cells of *L. monocytogenes* from heat pasteurization and provides for greater survival of this pathogen during PPTP treatments.

There was no significant (P > 0.05) interaction between pediocin concentration and pasteurization temperatures or heating times and no synergistic effect of these treatments was obvious. The impact of increased pasteurization temperature was generally greater than the impact of increased heating time within the limits of this study. Regardless of other factors, there was a significant (P < 0.05) difference between 71°C and 96°C pasteurization temperatures. The 81°C pasteurization temperature did not differ (P > 0.05) from either 71°C or 96°C. It was necessary to hold samples at 81°C or 96°C for at least 60 s to achieve a 50% reduction of initially inoculated *L. monocytogenes* numbers. Pasteurization at 71°C required at least 120 s to reach a 50% reduction of initially inoculated *L. monocytogenes* numbers. At the high inoculation level of *L. monocytogenes* (5.20 log CFU/g), most treatments resulted in some survival. Exceptions included samples treated with Pdn-6000 in single-link packages and immersed in hot water at 81°C for at least 120 s or at 96°C for at least 60 s (Fig. 1-C).

**Effect of package types on *L. monocytogenes***. PPTP treatments were clearly most effective (P < 0.001) in single-link packages (Fig. 1-A, 1-C), followed by 5-link packages. The 10-link packages resulted in the greatest (P < 0.001) *L. monocytogenes* survival. For frankfurters in single-link packages, the pathogen numbers were reduced by 3.0 logs or more for all but the two least severe time/temperature combinations.
Frankfurters in 5-link packages, exposed to 81°C for at least 60 s or 96°C for at least 30 s, achieved at least a 2.0-log reduction in pathogen populations. The numbers of *L. monocytogenes* on frankfurters in 10-link packages were reduced by about 1.5 to 2.5 log CFU/g, at best, even when a high pediocin concentration (Pdn-6000) was combined with pasteurization at 96°C for at least 60 s.

**Effect of treatment combinations on *L. monocytogenes***. Addition of pediocin (Pdn-3000 or Pdn-6000) reduced counts on samples inoculated with 3.40 log CFU/g by ca. 1.5 to 1.8 logs (13), and the thermal pasteurization resulted in further reductions of 1.5 to 3.0 logs and 1.6 to 3.4 logs, respectively. For the high inoculum (5.2 log CFU/g), addition of pediocin (Pdn-3000 or Pdn-6000) reduced counts by ca. 1.6 to 2.1 logs (13). In this case, the thermal pasteurization delivered additional effects to reach ca. 1.9- to 5.1-log and 2.3- to 5.2-log reduction in numbers, respectively.

The results indicated greater reduction of *L. monocytogenes* with the higher pasteurization temperatures, longer heating time and fewer frankfurters in the packages. With greater initial numbers of cells, longer heating time was necessary to cause complete destruction as might be expected. Pasteurization temperatures and package types had greater effects than heating time. While *L. monocytogenes* was reduced by all treatments, 81°C or greater for at least 60 s was necessary to achieve a 50% reduction of the initial inoculum. Addition of pediocin enhanced the antilisterial effects of PPTP treatments.

Aerobic plate counts (A. P. C.) for aerobic bacteria on TSA (Fig. 1-B and 1-D), resulted in similar survival patterns as observed with MOX agar. Most of the TSA counts were slightly higher than those on MOX agar. The colonies grown on TSA plates were predominantly *L. monocytogenes* as suggested by uniform appearance on both agars, typical of *Listeria* spp.. Though other organisms are included on TSA agar. However, some counts on TSA in these initial experiments were slightly lower than those on MOX agar. Therefore, we decided to use TSA plus 0.6% yeast extract (TSA-YE) for the remaining experiments, instead of TSA alone to improve the recovery of *L. monocytogenes*, particularly injured cells, during product storage.

**Evaluations during storage**

For evaluations during controlled temperature storage, we chose the treatments of Pdn-6000 combined with pasteurization temperatures of 81°C or 96°C, each for 60 s or 120 s. The objective was to compare the inhibitory effectiveness of these treatments during extended storage of frankfurters at 4°C, 10°C and 25°C. Frankfurters used for the storage studies were inoculated only at 3.40 log CFU/g with *L. monocytogenes*. All three package types were included.

**Survival and growth of *L. monocytogenes* on treated frankfurters stored at 4°C**

**Comparison of growth curves.** In Fig. 2-A, it is clear that pediocin alone, at either level, achieved an initial reduction of *L. monocytogenes* counts. The reduction was greater when pediocin was combined with heating treatments. No marked increases in populations of the control or the treated samples occurred during 12 weeks at 4°C. The *L. monocytogenes* counts for control samples at week 0 was 3.47 log CFU/g. The populations did not grow for 7 weeks. After week 7, the counts for all samples from selected treatments except
Figure 2. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging thermal pasteurization (PPTP) and stored at 4°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE) C. Treatments for frankfurters heated at 81°C or 96°C for 60 s or 120 s in 5-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in 1-link, 5-link and 10-link packages heated at 96°C for 120 s (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
05-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-PH-81-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 81°C for 60 s
05-PH-96-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 60 s
10-PH-96-120: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
01-PH-96-120: Frankfurters (1 link / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s

0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
for the singly packaged frankfurters submitted to the greatest time/temperature combination (96°C/120s),
appear to slowly increase. However, only the control samples increased significantly (P < 0.05) after 12 weeks.

**Effects of pasteurization temperatures and heating times.** Figure 2-C shows results for 5-link
packages only, treated with 6,000 AU pediocin and the PPTP treatments of 81°C/60s, 96°C/60s, and 96°C/120s,
relative to the control. The selected treatments were more (P < 0.05) effective than pediocin alone (Fig. 2-A)
during storage at 4°C. The growth curves for the populations following these 3 treatment combinations did not
differ greatly (P > 0.05). At week 0, the selected treatment combinations of pediocin and PPTP provided an
immediate reduction of the *L. monocytogenes* numbers to 0.46 to 1.11 log CFU/g, compared to the control at
3.47 log CFU/g. In addition, delayed growth of this pathogen in these selected treatments was evident up to 7
weeks. After storage for 7 weeks, the populations appear to increase but the increase was not significant (P >
0.05).

The pasteurization temperature at 96°C was significantly (P < 0.05) more effective than 81°C.
However, there was no significant (P > 0.05) difference between 60 s and 120 s at 96°C. In general,
pasteurization temperatures had a greater effect than heating times for PPTP treatments and this effect continued
during storage.

**Effects of package types.** Comparison of the results from the different package types (single-link, 5-
link and 10-link packages) treated with 6,000 AU pediocin, pasteurized with the 96°C/120s treatment and stored
at 4°C is shown in Fig. 2-D. At week 0, all three package types provided an immediate reduction of *L.
monocytogenes* counts to 0.46 to 0.80 log CFU/g. Frankfurters in single-link packages treated with the
96°C/120s treatment showed little or no growth throughout the 12-week storage period. The *L. monocytogenes*
populations for all of the other treatments were able to recover and grow after 7 to 8 weeks. However,
populations remained lower (maximum of 2.10 log CFU/g) throughout storage than the original inoculation
level (3.47 log CFU/g) of the control. The results indicate that with fewer links of frankfurters per package,
PPTP treatments were more effective.

Plating on either MOX agar or TSA-YE produced comparable results leading to similar conclusions
(Fig. 2-A and 2-B). It was observed that the treatments that inhibited or slowed growth of *L. monocytogenes* as
determined on MOX agar gave the same results using TSA-YE, a medium that enhances recovery of the
pathogen because it lacks the selective antimicrobials included with MOX agar.

**Survival and growth of *L. monocytogenes* on treated frankfurters stored at 10°C**

**Comparison of growth curves.** For samples stored at 10°C, the *L. monocytogenes* populations on the
control began to increase immediately after week 0 (3.38 log CFU/g), and became significantly (P < 0.05)
greater at week 6 (5.70 log CFU/g) (Fig. 3).

In Fig. 3-A, the selected treatments fall into two categories similar to results at 4°C. Pediocin alone
reduced *L. monocytogenes* counts initially. The combinations of pediocin and PPTP achieved greater (P < 0.05)
initial reduction of *L. monocytogenes* counts than pediocin alone and the control, except for the 5-link/80°C/60s
Figure 3. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging thermal pasteurization (PPTP) and stored at 10°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE) C. Treatments for frankfurters heated at 81°C or 96°C for 60 s or 120 s in 5-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in 1-link, 5-link and 10-link packages heated at 96°C for 120 s (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
05-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-PH-81-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 81°C for 60 s
05-PH-96-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 60 s
10-PH-96-120: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
05-PH-96-120: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
01-PH-96-120: Frankfurters (1 link / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
After delayed growth of 1-2 weeks, most of the treated samples showed recovery and relatively rapid growth. Only the most severe PPTP treatment (96°C/120s) combined with pediocin showed growth suppression during 12 weeks of storage.

**Effects of pasteurization temperatures and heating times.** Figure 3-C shows the results of 6,000 AU pediocin and the PPTP treatments (81°C/60s, 96°C/60s, and 96°C/120s) for 5-link packages. Growth appears to be delayed by 1 to 2 weeks and populations steadily increased thereafter. There were significant (P < 0.05) differences at specific sampling times among these 3 treatments. The 5-link/96°C/120s treatments had significantly (P < 0.05) lower counts than the 5-link/81°C/60s treatment during the first 1.5 weeks. However, after storage for 1.5 weeks at 10°C, there was no significant (P > 0.05) difference among these 3 treatments. Throughout storage for 12 weeks, the antilisterial effectiveness of the 96°C pasteurization temperature did not differ significantly (P > 0.05) from 81°C. However, there was also no significant (P > 0.05) difference between the heating times (60 s and 120 s) at 96°C throughout storage for 12 weeks.

**Effects of package types.** Figure 3-D compares the antilisterial effect of different package types treated with 6,000 AU pediocin, pasteurized with the 96°C/120s treatment and stored at 10°C. The antilisterial effects of the single-link packages was significantly (P < 0.05) greater than for 5-link and 10-link packages, which had significantly (P < 0.05) lower counts than the control. There was no significant (P > 0.05) difference between the latter two package types. The results indicate that fewer links of frankfurters in packages submitted to PPTP treatments resulted in fewer survivors of L. monocytogenes. Delayed growth of the pathogen on samples in the 1-link/96°C/120s treatment is evident. There was a delay in growth of 3 to 4 weeks for single-link packages compared to 1 to 2 weeks for the 5-link and 10-link packages. After storage for 3.5 weeks, the pathogen in single-link packages still did not show a significant (P > 0.05) increase, though some survivors recovered. The population did not exceed 2.31 log CFU/g, throughout storage for 12 weeks and remained significantly (P < 0.05) lower than the other treatments. Samples in 5-link/96°C/120s packages showed significantly (P < 0.05) less survivors than those in 10-link/96°C/120s packages during the 1 to 2 week lag phase. However, the antilisterial effects of treatments in both the 5-link and 10-link packages were evident (P < 0.05) compared to the growth of L. monocytogenes in control samples during storage for 12 weeks. The population in the treated 5-link and 10-link packages remained significantly (P < 0.05) lower than controls for up to 6 weeks.

The populations of L. monocytogenes on TSA-YE (Fig. 3-B) were generally similar or slightly higher than those on MOX agar (Fig. 3-A). However, the populations of this pathogen measured on TSA-YE from frankfurters in 1-link/96°C/120s packages were clearly greater than those measured on MOX agar. These differences probably reflect the greater recovery of a large number of injured cells when using TSA-YE. However, even with improved recovery, the populations of the pathogen did not exceed 3.25 log CFU/g in the singly-package samples during storage for 12 weeks.
Survival and growth of *L. monocytogenes* on treated frankfurters stored at 25°C

**Comparison of growth curves.** With storage at 25°C, *L. monocytogenes* grew within 2 to 3 days for all but one treatment (Fig. 4). After 3 days, counts of most of the samples reached the maximum levels (5.85 log CFU/g). In Fig. 4-A, the treatments that combined pediocin with PPTP resulted in greatest effectiveness relative to the control. Samples treated with pediocin alone were intermediate in effectiveness. The growth profile and treatment effects were generally similar at 25°C to those observed with storage at 4°C and 10°C. The 1-link/96°C/120s treatment was significantly (P < 0.05) different from the others with greater obvious inhibition during the storage period.

**Effects of pasteurization temperatures and heating times.** Figure 4-C shows the antilisterial effectiveness in 5-link packages of Pdn-6000 and selected PPTP treatments (81°C/60s, 96°C/60s, and 96°C/120s) during storage at 25°C. Only a slight delay in growth of the pathogen occurred on frankfurters during the first 1 or 2 days and populations steadily increased thereafter. Throughout storage for 10 days, the populations of *L. monocytogenes* on samples heated at 81°C appeared to be greater than those of samples heated at 96°C, but these differences were not significant (P < 0.05). Again, there was no significant (P > 0.05) difference between 60 s and 120 s at 96°C.

**Effects of package types.** Figure 4-D shows the comparison for antilisterial effectiveness of the different package types of Pdn-6000 and the heat treatment of 96°C/120s and stored at 25°C. The single link package clearly resulted in greater effectiveness for the PPTP treatment. Interestingly, in this case, the populations on samples in 5-link packages were somewhat greater than those in 10-link packages after day 0 and during storage.

Growth of *L. monocytogenes* in 5-link and 10-link packages was much more rapid on frankfurters during storage at 25°C than at 4°C or 10°C as expected. After storage for 3 to 5 days, the counts increased significantly (P < 0.05) to 3.02 to 4.01 log CFU/g, and within 6 to 10 days, the populations reached a maximum level of 5.22 to 5.77 log CFU/g, respectively. Frankfurters in the single-link packages remained below 2.75 log CFU/g throughout storage for 12 days.

The populations of *L. monocytogenes* measured on TSA-YE (Fig. 4-B) were similar or slightly higher than those on MOX agar (Fig. 4-A). However, again the populations on frankfurters in 1-link/96°C/120s packages measured on TSA-YE were clearly greater than those on MOX agar. This was particularly so after storage for 10 days. The greater number may be due to the recovery and growth of background microorganisms, such as spore-forming bacteria that are highly heat-tolerant.

**Storage temperature effects.** As expected, growth of *L. monocytogenes* was more rapid on treated frankfurters stored at 10°C (Fig. 3-A) for 12 weeks than on those stored at 4°C (Fig. 2-A) and was most rapid at 25°C (Fig. 4-A). The population reduction of the pathogen on treated frankfurters was similar following the heat treatments but growth during storage was largely dependent on storage temperatures used in this study. Overall effects of the antilisterial treatments subjected to the three holding temperatures can be summarized as
Figure 4. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging thermal pasteurization (PPTP) and stored at 25°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE) C. Treatments for frankfurters heated at 81°C or 96°C for 60 s or 120 s in 5-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in 1-link, 5-link and 10-link packages heated at 96°C for 120 s (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
05-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-PHI-81-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 81°C for 60 s
05-PHI-96-60: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 60 s
10-PHI-96-120: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
05-PHI-96-120: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s
01-PHI-96-120: Frankfurters (1 link / pkg) treated with 6,000 AU pediocin and immersed in hot water at 96°C for 120 s

0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
follows; while the initial reduction in *L. monocytogenes* numbers depended on the addition of pediocin and the thermal treatment, the length of the subsequent lag phase was dependent on the storage temperatures. Thermal pasteurization effects were, as noted earlier, very dependent upon package types with the order of effectiveness being single-link > 5-link > 10-link packages. Temperature of the PPTP, particularly at 96°C, was more important than time for the pasteurization conditions studied.

**Product quality analyses**

Based on the microbiological results, the most effective treatment combinations were evaluated for potential product quality changes that might be introduced by the treatments. The treatments selected for sensory evaluations included the frankfurters treated with 6,000 AU of pediocin and heated at 81°C or 96°C, each for 60 s. The 96°C treatment for 60 s was chosen because it provided equivalent antilisterial effectiveness as 96°C for 120 s and would be more practical to implement.

**Effects of selected treatments on quality of frankfurters.** The results for purge, color, texture, pH and oxidative change (TBA values) are presented in Table 1. There was no effect of treatment on package purge except for the volumes of solutions added to packages. The addition of pediocin, for example, at 1 ml or 2 ml per frankfurter resulted in a significant increase in the water measured as purge. The purge accumulation for samples treated with Pdn-6000 and PPTP was 5.35 to 5.76% compared to control samples (0.61%). However, the PPTP treatments did not increase the amount of measured purge by a significant amount over that of packages with pediocin (Pdn-6000) that did not receive a post-packaging heat treatment.

Color values of the frankfurters were modified by the PPTP treatments (Table 1). In this study, the pasteurization treatments resulted in color which was darker (L* value) and redder (a* value) than controls. These differences were not large but were statistically significant. Texture characteristics, either exterior skin toughness or interior firmness, were unaffected by any of the treatments. There was no significant (P > 0.05) difference for Hunter b* values, firmness of skin and interior, or TBA values. The results showed that pH values were also influenced by addition of 40% ALTA™ 2341 suspension, though the decrease in pH was small. After treatments, the samples with pediocin were darker, redder and more acidic than control samples.

**Effects of pasteurization temperatures and times on purge accumulation of frankfurters.** Figure 5 shows that PPTP treatments without added pediocin solution or inoculum solution had no (P > 0.05) effect on purge. The frankfurters heated at 81°C and 96°C for 60 s and 120 s had little purge accumulation and were similar to control samples. These results indicate that the purge accumulation measured in inoculated samples after treatments was primarily from addition of 40% ALTA™ 2341 suspension and *L. monocytogenes* inoculum, not from the frankfurters themselves. However, package types affected purge as shown in Fig. 5.

**Effects of selected treatments on sensory quality of frankfurters.** Sensory panelists evaluated frankfurters for visual purge, color, texture and specific preselected aroma notes. The results are shown in Table 2. The purge assessment again reflects the volume of added solutions such as the 1 ml (Pdn-3000) or 2 ml (Pdn-6000) of pediocin. The control was significantly (P < 0.05) lower for apparent purge than the treatments because there was no added solution. There was no significant (P < 0.05) difference in purge as a
Table 1. Comparison of physical and chemical analyses of frankfurters following selected treatments with pediocin (in ALTA™ 2341) and post-packaging thermal pasteurization (PPTP)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge (%)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Firmness (kg) Skin</th>
<th>Firmness (kg) Interior</th>
<th>pH</th>
<th>TBA (mg MDA/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.61 ± 0.25</td>
<td>44.98 ± 0.90</td>
<td>15.14 ± 1.12</td>
<td>13.67 ± 0.90</td>
<td>0.40 ± 0.03</td>
<td>0.20 ± 0.01</td>
<td>6.12 ± 0.02</td>
<td>0.64 ± 0.07</td>
</tr>
<tr>
<td>Pdn-30001</td>
<td>3.33 ± 0.62</td>
<td>44.38 ± 1.50</td>
<td>15.76 ± 0.97</td>
<td>13.35 ± 0.71</td>
<td>0.40 ± 0.04</td>
<td>0.20 ± 0.01</td>
<td>6.08 ± 0.01</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>Pdn-60002</td>
<td>5.13 ± 0.67</td>
<td>42.93 ± 1.03</td>
<td>15.93 ± 1.41</td>
<td>13.19 ± 0.49</td>
<td>0.40 ± 0.03</td>
<td>0.21 ± 0.01</td>
<td>6.05 ± 0.05</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>PH-81-603</td>
<td>5.76 ± 0.96</td>
<td>42.85 ± 2.16</td>
<td>16.05 ± 1.27</td>
<td>13.02 ± 0.66</td>
<td>0.41 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>6.05 ± 0.06</td>
<td>0.61 ± 0.06</td>
</tr>
<tr>
<td>PH-96-604</td>
<td>5.35 ± 1.02</td>
<td>42.32 ± 1.13</td>
<td>16.47 ± 0.60</td>
<td>13.10 ± 0.21</td>
<td>0.46 ± 0.03</td>
<td>0.23 ± 0.01</td>
<td>6.02 ± 0.01</td>
<td>0.79 ± 0.12</td>
</tr>
</tbody>
</table>

1. Pdn-3000: Frankfurters (5 links / pkg) treated with 3000 AU pediocin
2. Pdn-6000: Frankfurters (5 links / pkg) treated with 6000 AU pediocin
3. PH-81-60: Frankfurters (5 links / pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 s
4. PH-96-60: Frankfurters (5 links / pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 s

Means within a column with different letters a, b, c are not significantly (P < 0.05) different
Figure 5. Purge accumulation (%) of frankfurters after post-packaging thermal pasteurization

PH-81-60: Frankfurters (1, 5 & 10 link[s] / pkg) immersed in hot water at 81°C for 60 s.
PH-81-120: Frankfurters (1, 5 & 10 link[s] / pkg) immersed in hot water at 81°C for 120 s.
PH-96-60: Frankfurters (1, 5 & 10 link[s] / pkg) immersed in hot water at 96°C for 60 s.
PH-96-120: Frankfurters (1, 5 & 10 link[s] / pkg) immersed in hot water at 96°C for 120 s.
Table 2. Sensory evaluation of frankfurters following selected treatments with pediocin (in ALTA™ 2341) and post-packaging thermal pasteurization (PPTP)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge</th>
<th>Color</th>
<th>Texture</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Smoky</td>
<td>Burnt</td>
</tr>
<tr>
<td>Control</td>
<td>1.29 ± 0.17</td>
<td>5.98 ± 0.55</td>
<td>7.10 ± 0.95</td>
<td>7.04 ± 0.50</td>
</tr>
<tr>
<td>Pdn-3000 ²</td>
<td>5.41 ± 1.73</td>
<td>6.99 ± 1.67</td>
<td>8.12 ± 1.03</td>
<td>7.46 ± 0.41</td>
</tr>
<tr>
<td>Pdn-6000 ³</td>
<td>9.19 ± 0.39</td>
<td>8.10 ± 1.42</td>
<td>9.50 ± 1.14</td>
<td>7.41 ± 0.53</td>
</tr>
<tr>
<td>PH-81-60 ⁴</td>
<td>8.54 ± 0.63</td>
<td>8.06 ± 0.54</td>
<td>9.68 ± 0.51</td>
<td>6.60 ± 0.64</td>
</tr>
<tr>
<td>PH-96-60 ⁵</td>
<td>8.64 ± 0.36</td>
<td>7.77 ± 0.39</td>
<td>9.50 ± 0.69</td>
<td>7.36 ± 0.64</td>
</tr>
</tbody>
</table>

1. The numerical scales of sensory intensity use for purge, color, texture, aroma of smoky, burnt and acid were: 0 = none, extremely light, extremely soft, and none, respectively; and 15 = extremely abundant, dark, firm, and intense, respectively.

2. Pdn-3000: Frankfurters (5 links / pkg) treated with 3000 AU pediocin

3. Pdn-6000: Frankfurters (5 links / pkg) treated with 6000 AU pediocin

4. PH-81-60: Frankfurters (5 links / pkg) treated with 6000 AU pediocin and heated in hot water at 81°C for 60 s

5. PH-96-60: Frankfurters (5 links / pkg) treated with 6000 AU pediocin and heated in hot water at 96°C for 60 s

Means within a column with different letters a-c are not significantly (P < 0.05) different.
result of the PPTP treatments.

The trend of color scores (Table 2) was lower for the control group compared with all treatments but this was not significant. Odor scores, evaluated as smoky, burnt and acidic aroma, did not differ. The texture assessments by the sensory panel indicated increased force was necessary to cut the frankfurters with the edge of a fork in the case of the PPTP treatments (Table 2). The addition of pediocin also increased the product resistance to cutting and was significant at the higher level of addition (Pdn-6000). This effect was surprising and a potential explanation is not clear. Instrumental evaluations of texture, however, showed no significant (P < 0.05) differences among the treatments or between the treatments and the control for either skin toughness or interior firmness of the frankfurters (Table 1). Except for the purge accumulation, panelists could not detect significant (P> 0.05) differences between samples after treatments. The results showed that the PPTP treatments evaluated did not appreciably alter the scores for color (7.77-8.06), texture (9.50-9.68) or aroma which included smoky (6.60-7.36), burnt (1.65-2.30) and acidic (4.78-5.21) notes compared to control samples.

Discussion

Inhibitory effects of PPTP treatments and other factors on *L. monocytogenes*

**Antilisterial effect of PPTP treatments.** The use of adequate heat treatment to destroy pathogenic and spoilage microorganisms is one of the most effective food-preservation processes in use today and has been used for centuries (24). It has been reported by Zaika et al. (54) that cooking frankfurters to an internal temperature of 71°C (160°F) should kill *L. monocytogenes* if present at 10^3 CFU/g or less. Therefore, heat treatment is a CCP in RTE meat processing to assure microbial safety relative to *L. monocytogenes*.

**Antilisterial effect of pasteurization temperatures.** PPTP treatments have been shown to be an effective microbial intervention method to eliminate *L. monocytogenes* and other indigenous microorganisms on precooked packaged beef chunks and RTE deli-style meats (14,40). The results from our study show similar effects for frankfurters and are consistent with those studies. It was found that pasteurization temperature at 96°C was the most effective temperature used in this study to inhibit the growth of *L. monocytogenes*. In our study, there were 1.5- to 3.4- or 1.9- to 5.2-log reductions of *L. monocytogenes* on frankfurters with the different inoculation levels (3.40 or 5.20 log CFU/g), respectively, depending on package types. *L. monocytogenes* on meat products has been reported to survive heating at 80°C (34) and even as high as 99°C (45). According to these studies, the reduction of the pathogen on PPTP treated meat products did not exceed 4.5 logs. According to other studies (21, 40, 45), 90.6°C to 99.0°C has been reported be the more effective temperature range to inactivate this pathogen. However, in our study, complete reduction of 3.40 or 5.20 logs was observed in our initial evaluation where pediocin enhanced the antilisterial effectiveness of PPTP treatments.

Muriana et al. (40) reported that heating time of 2 min at 90.6°C to 96.1°C provided 2-log reduction of *L. monocytogenes* in most of RTE deli meats and 4-log reduction if heated for 10 min. A variety of large-sized (5 to 12 lb) RTE meat products, including turkey, ham, and roast beef, were evaluated. The population reduction reported by Muriana et al. (40) was slightly less than that for frankfurters in 10-link packages from
our study. Muriana et al. (40) suggested that \textit{L. monocytogenes} cells (~ $10^7$ CFU/g) infiltrated irregular areas, such as surface cuts, folds, grooves, and skin, and were protected as a result. These surface imperfections resemble the interfacial areas between frankfurters in multiple-link packages. These areas located away from the package surface will shield bacteria from the effects of the heat process. Pediocin may provide the additional inhibition needed to overcome the protective cover of internal product surfaces.

\textbf{Antilisterial effect of heating times.} Samelis et al. (47) found that the initial inoculation numbers of \textit{L. monocytogenes} (3.9 log CFU/cm$^2$) on frankfurters were reduced in proportional to heating times. Counts in single-link or 2-link packages proportionally decreased with increasing heating times from 30 s to 90 s at 75°C or 85°C. Similar results were observed in our study in that as the heating times increased from 30 s to 120 s, antilisterial effectiveness at 71°C, 81°C or 96°C was enhanced.

\textbf{Antilisterial effect of PPTP combinations.} It is evident that pasteurization temperatures and heating times are both important considerations to inactivate \textit{L. monocytogenes} on RTE meats. The reduction of \textit{L. monocytogenes} has been reported to be proportional to both the immersion pasteurization temperatures and heating times (47). According to the results from our data, the impact of heating temperature was much greater than that of immersion heating times. The results from our study suggested that PPTP at 96°C for at least 60 s was necessary to reduce a large proportion of \textit{L. monocytogenes} on frankfurters in packages. In a study of PPTP treatments alone, a reduction of 4.5 logs of \textit{L. monocytogenes} inoculated on precooked beef roasts was reported for heating at 96°C for 5 min (21). Additionally, \textit{L. monocytogenes} ($10^8$ CFU/ml) on chubs of summer sausage, vacuum-sealed in bags, showed ca. 3-log reduction after heating at 99°C, 88°C or 77°C for at least 30 s, 60 s, or 90 s, respectively (45).

\textbf{Antilisterial effect of pediocin (in ALTA™ 2341).} Generally speaking, Pdn-6000 (6,000 AU pediocin), which was the maximum concentration used in this study, reduced \textit{L. monocytogenes} counts but may be insufficient alone for adequate control of \textit{L. monocytogenes} on RTE meat products. However, the pediocin concentration in commercial fermentation products such as ALTA™ 2341 is relatively low and use of more highly purified pediocin with greater product concentrations may improve the antilisterial effects on RTE meat products.

\textbf{Synergistic effect of pediocin and PPTP in single-link packages.} The single-link packages, where treatments of pediocin and PPTP had the greatest effects on \textit{L. monocytogenes}, showed little or no growth when stored at 4°C or 10°C for 12 weeks, or 25°C for 12 days. This is a good example of multiple hurdle technologies. It has been reported that nisin enhanced the effectiveness of thermal processing during mild heat treatments compared with heat or nisin treatment alone (9, 24). Addition of nisin at various concentrations rendered \textit{L. monocytogenes} sensitive to the lethal effect of heat (24). A synergistic effect between moderate, sublethal heat (52°C for 3 min) and nisin enhanced the inactivation of \textit{L. monocytogenes} (35) on turkey skin and in the scald water used for turkey processing. These authors also suggested that refrigeration (4°C) had a synergistic effect with heat and nisin.
Montville and Chen (39) indicated that both nisin and pediocin (pediocin PA-1) are lactic acid bacteria (LAB) bacteriocins that share some common modes of action to act primarily at the cytoplasmic membranes of susceptible microorganisms. Nisin and pediocin (pediocin AcH) reduced the viability of cells surviving sublethal stresses, such heating at 55°C for 10 min (26). These authors suggested that both bacteriocins are likely to enter the bacterial cell walls (or outer membranes) to destabilize the cytoplasmic (or inner) membranes and kill cells of sensitive gram-positive and resistant, but injured, gram-negative and gram-positive bacteria. This is also suggested by the results of our study, especially from packages containing single-link frankfurters. The effect of PPTP/pediocin combinations was much greater than that of pediocin alone. The reduction of \textit{L. monocytogenes} counts was greater with increased pediocin concentrations (Pdn-6000 > Pdn-3000) and heating times (120 s > 60 s > 30 s), especially at the lower pasteurization temperature (71°C). For example, for single-link packages inoculated with 5.20 log CFU/g and heated at 71°C, the reduction of counts with Pdn-6000 were greater than for those with Pdn-3000.

**Factors affecting the antilisterial effectiveness of package types**

**Effect of package types.** The package type is an important consideration for PPTP inactivation of \textit{L. monocytogenes} on frankfurters. The results from single-link packages after treatments showed synergistic effects between pediocin and PPTP treatments. Samelis et al. (47) reported that single-link frankfurters in packages heated at 75°C or 80°C for 90 s provided immediate reduction of the pathogen. Counts remained lower (2.1 logs) than the initial inoculation level (3.9 logs) of the unheated controls throughout 50 days of storage at 4°C. Our results were similar for single-link packages. However, little or no synergistic effect was observed in the larger multiple-link packages when interfacial areas shielded cells from the heat process. The results from initial evaluations of our study indicated that there were significant (P < 0.05) differences between single-link packages and 10-link packages. This difference was more evident with increased pasteurization temperature, heating time and pediocin concentration. Most sample packages showed more survivors in samples at the center area of the package compared with those from the outside area (data not shown). It is very clear that the greater interfacial areas in 10-link packages decreased the heat exposure, and created more space to shield or protect the pathogen from effects of heating.

Despite the observed reduction in \textit{L. monocytogenes} populations at day 0 or week 0 due to heat treatments, the organism was able to recover and grow in most samples during storage. Relatively few treatments reduced the \textit{L. monocytogenes} numbers to undetectable levels. The greater interfacial areas within packages and possibly, heat shock-response contributed to more of the pathogen surviving in 5-link and 10-link packages. Juneja (24) indicated that sublethal heat stress may render \textit{L. monocytogenes} more resistant to subsequent lethal heat treatments. Heat-shock response and induced thermotolerance has been reported in a wide range of microorganisms including \textit{L. monocytogenes} (20, 29, 30). Generally speaking, heat-shocked cells of \textit{L. monocytogenes} may need to be heated twice as long as non-heat-shocked cells to achieve the same extent of lethality (18). Heat shock proteins (HSPs), induced by heat-shock response, may enhance the survival of \textit{L. monocytogenes} in meats during exposure to subsequent temperature treatments (24, 50).
It was observed that frankfurters stored at 25°C in our study resulted in \textit{L. monocytogenes} populations in the 10-link/96°C/120s treatment that remained lower than that for the 5-link/96°C/120s treatment. This situation may be due to stimulation of the heat-shock response to induce HSPs and protective effects in the 5-link packages. Because the 5-link packages experienced more severe exposure to the heat process than 10-link packages, we postulated that the survivors may have received greater heat-shock stimulation to repair their damage when samples were stored at 25°C. The surviving populations of \textit{L. monocytogenes} on samples in 5-link packages recovered and grew more rapidly than those for samples in 10-link packages. It may be that the repair systems or enzymes of \textit{L. monocytogenes} can be induced faster and more effectively in this situation, particularly when samples are stored at higher temperatures, such as 25°C. It has been reported that abusive temperature, such as 10°C, provided a more conductive environment to repair sublethally injured cells of this pathogen (21).

\textbf{Effect of cold purge from interior of products.} Cold purge that is generated from frankfurters by packaging shrinkage and which migrates from the cold product interior to product surface may counteract some of the heat effects of PPTP treatments (40). Therefore, inactivation of \textit{L. monocytogenes} may be less than expected. The data from Table 1 indicated that the apparent purge accumulation (%) from samples treated with Pdn-6000 and heated at 81°C or 96°C, each for 60 s, was not significantly (\(P > 0.05\)) different from samples treated with Pdn-6000 alone. Muriana et al. (40) suggested that the migration of purge from the chilled (2°C to 4°C) interior to the surface reduced the overall heating effect and concomitant pathogen reduction. They found that with heating for as long as 10 min at 96.1°C, the interior temperatures of samples did not change. Heat penetration occurred only in the outer 1 cm of sample, and surface temperatures equilibrated back to below 12°C within a few min after cool-down in a chill tank. This may explain why there was no dramatic (\(P > 0.05\)) difference in our study between 60 s and 120 s heating time, when samples were heated at 96°C.

\textbf{Effect of curing ingredients.} Several studies have reported that the combinations of temperature, pH, salt, and sodium pyrophosphate at adequate levels can lower thermal resistance of microorganisms (8, 25, 37). Sodium nitrite may protect cured meat against \textit{L. monocytogenes}, particularly if employed in combination with acidic pH (pH 6.0 or below), vacuum packaging, high salt concentrations, and adequate refrigeration (8, 34, 37). These combinations of ingredients are sublethal stresses for the survivors and may increase their tolerance for environmental stresses, including subsequent heat. \textit{L. monocytogenes} cells have been shown to survive after treatments with curing ingredients mentioned above, and the survivors produced HSPs (24, 50). Hence, it is likely that survivors in multiple-link packages induced similar heat-shock response with the partial protection provided by the multiple-link packages. The survivors may become tolerance and adapt to the new conditions after exposure to these sublethal stresses. This kind of adaptation can improve the survival of \textit{L. monocytogenes} when exposed to other sublethal stresses (31, 32).

\textbf{Effect of pasteurization types.} Several studies have reported that surface pasteurization of RTE products was effective for inactivation of pathogens when done with steam prior to packaging (14, 15, 21, 27). However, in this case, there is still a risk of post-processing contamination before sealing packages. We are
suggesting that PPTP treatments may be a better choice for assured control of *L. monocytogenes* on RTE products.

**Effect of storage temperatures.** The results from our study demonstrated that refrigeration temperature (4°C) can slow the growth of *L. monocytogenes* on frankfurters treated with pediocin and PPTP. However, this pathogen still can revive and multiply slowly at 4°C. With the multiple hurdle combinations, *L. monocytogenes* counts on frankfurter were reduced, but not eliminated. It has been reported by others that *L. monocytogenes* on inoculated ground beef (> 10^5 CFU/g) and frankfurters (3.9 log CFU/cm^2) survived and subsequently proliferated during storage at 4°C after heat treatments (7, 47).

**Changes in product quality after treatments**

**Effect of PPTP treatments on product quality.** In general, severe heat treatment is likely to impair the organoleptic properties and may even affect nutritional values of foods. To avoid undesirable effects of heat, mild heating in combination with other preservation treatments to enhance preservative effectiveness by additive or synergistic effects is preferred (24). Muriana et al. (40) demonstrated that with PPTP at 90.6°C to 96.1°C for 2 min, the outer 1 cm of the product increased in temperature with minimal effects on product appearance or quality. They found surface temperatures returned to below 50°F (12°C) within a few minutes after cooling in a chill tank. Hardin et al. (21) indicated that the greatest increase in temperature for all treatments, which included precooked beef roasts (< 2 lb) exposed to 91°C or 96°C for 3 or 5 min, at both the surface and 3 mm below, occurred within the first minute. After this time, temperature remained constant for all treatments. The results from our data for sensory evaluations showed no negative quality effects on the products.

Roering et al. (45) indicated that PPTP at temperatures of 77°C or greater for 60 s to 120 s was sufficient to inactivate appreciable numbers of *L. monocytogenes* and did not cause appreciable quality changes, such as “greasing out” or textural changes, in the finished product. Further, the treatments mentioned above did not appreciably change the flavor or color of the finished product.

**Effect of pediocin and PPTP on physical and chemical attributes.** The changes in quality attributes of frankfurters following selected treatments with pediocin and PPTP were similar to those for samples treated with pediocin alone (13). The addition of the external solution with pediocin resulted in differences for apparent purge accumulation, color, and pH values of frankfurters. The sensory panelists scored texture of frankfurters treated with PPTP and Pdn-6000 higher in firmness (P < 0.05) compared to control samples, but this was not confirmed by the instrumental texture measurements. This suggests that textural changes were relatively small.

**Conclusions**

Generally, the treatment combinations used in this study can be practically applied in the meat industry as one of the CCPs in a HACCP plan without major concerns for product quality changes. This study effectively demonstrated the concept of multiple hurdle technology (36) as a good means to control *L.*
monocytogenes on RTE processed meat products, such as frankfurters. The multiple hurdles combined at lower levels were more effective than each single treatment at high levels, similar to reports by others (28, 51). Adding pediocin solution (physicochemical barrier) to the surface of frankfurters (pre-packaging treatment) directly contacted cells of L. monocytogenes and lowered the initial populations of the pathogen. Therefore, PPTP treatments could more easily inactivate or inhibit growth of the remaining pathogens after sealing packages without chances for reintroduction of L. monocytogenes. This process could prove to be a significant additional hurdle to help in providing safer RTE deli products. The PPTP concept already has been integrated into processing lines of various companies to implement the hurdle concept and reduce the surface contamination of L. monocytogenes on RTE deli meat products. The results of our study support this suggestion and in addition suggest that pediocin could be used as another hurdle in the process.

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References


CHAPTER 5. EFFECTS OF PEDIOCIN AND POST-PACKAGING IRRADIATION PASTEURIZATION ON LISTERIA MONOCYTOGENES ON FRANKFURTERS

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Abstract

Frankfurters, in single-link, 5-link or 10-link packages, were surface-inoculated with a 5-strain mixture of *Listeria monocytogenes* (3.40 or 5.20 log CFU/g) after treatment with 3,000 AU or 6,000 AU pediocin (in ALTA™ 2341) per link (Pdn-3000 or Pdn-6000). The frankfurters were vacuum-packaged, after which the single-link and 5-link packages were irradiated at 1.2 or 2.3 kGy and the 10-link packages were irradiated at 1.4 or 3.5 kGy. *L. monocytogenes* was enumerated following the treatments. Selected treatments were subsequently evaluated during storage at 4°C, 10°C and 25°C for up to 12 weeks.

A combination of pediocin (at least 3,000 AU) and post-packaging irradiation at 1.2 kGy or more was necessary to achieve a 50 % reduction of *L. monocytogenes* on frankfurters in single-link or 5-link packages. The combination of 6,000 AU of pediocin and irradiation of 2.3 kGy or more was effective in all package types for inhibition of the pathogen for 12 weeks at 4°C or 10°C, and single-link packages retained antilisterial effectiveness for 12 days at 25°C. There was a synergistic effect between pediocin and irradiation for inhibition of *L. monocytogenes*. Storage at 4°C enhanced the antilisterial effects of the pediocin/irradiation combinations, with little or no growth of the pathogen in single-link or 5-link packages during 12 weeks of storage. In general, these treatments did not affect the sensory quality of frankfurters.

Introduction

*L. monocytogenes* can survive at refrigeration temperatures and applying low-temperature storage alone cannot be relied upon to keep meat safe from this organism (21). Moreover, the pathogen has been reported to persist in meat processing environments, even after rigorous cleaning and sanitation (26). Even in well-managed processing plants, cooked products may become contaminated before packaging; several such cases have been documented (33). The presence of *L. monocytogenes* in processing environments renders post-processed foods at risk for contamination before or during packaging (29). New intervention strategies are needed to ensure inactivation of *L. monocytogenes* on ready-to-eat (RTE) meat products (4). Antimicrobial treatments such as pediocin (22), post-packaging thermal pasteurization (25) or irradiation pasteurization (12, 13, 28) are effective intervention means to improve control *L. monocytogenes* on meat products.

Cells of *L. monocytogenes* can be injured from sublethal stresses, but may not be killed. Subsequently, these injured cells may recover and become more resistant to further antimicrobial treatments. Biofilm
formation, for example, that will protect colonies from cleaning and sanitation treatments (7, 32) has been observed. Taormina and Beuchat (29) suggested that alkaline-adapted cells that survived subsequent exposure to sanitizers would not be inhibited on vacuum-packaged frankfurters stored at refrigeration temperatures. Bower and Daeschel (7) indicated that applying multiple hurdle preservation approaches properly is one of the major strategies to counter resistance development by pathogens and effectively control them.

Adequate absorbed doses of irradiation will effectively kill disease-causing pathogens, including *L. monocytogenes*, and delay food spoilage (20). Fu et al. (13) suggested that irradiation provides a method to reduce dependence on chemical additives because it decreases the load of microorganisms and eliminates some food pathogens. Low-dose to medium-dose, ≤ ca. 3 kGy, irradiation has become available to control pathogens in meat products (15, 24) and offers the potential to minimize changes in finished product characteristics at the same time (24). Fu et al. (13) reported that medium-dose irradiation at 2.0 kGy resulted in a reduction of almost 6-log of *L. monocytogenes* on cured ham. However, this dose did not eradicate *L. monocytogenes* because some cells were able to recover when the temperature was elevated to 25°C. These authors suggested that lower refrigeration temperatures contributed to growth inhibition of *L. monocytogenes*. Fu et al. (12) reported that irradiation reduced the numbers of the pathogen populations by 3 and 5 logs with doses of 1.5 kGy for steaks and 2.0 kGy for ground beef, respectively. Storage at 7°C was effective for suppressing growth of this pathogen because numbers increased only 1 log on both nonirradiated and irradiated (0.9 kGy) cured ham (13). The authors suggested that addition of nitrite to ham contributed to inhibition of *L. monocytogenes*. Another study reported that the microbiological shelf-life of pork loin irradiated at 3.0 kGy was extended to over 90 days at 2°C to 4°C compared to 41 days for nonirradiated samples (14). Radomyski et al. (23) suggested that food irradiation should be considered as a major technology to meet the safety demands of consumers in the present and future meat and poultry markets.

Pediocin has been used successfully as an antimicrobial treatment to control the growth of *L. monocytogenes* (8, 22) in a variety of meats (17, 18, 19) including frankfurters (5) and wieners (11). Applying multiple hurdle preservation approaches, such as using pediocin in conjunction with post-packaging irradiation and low temperature storage, may be considered a good strategy to effectively control post-processing contamination by *L. monocytogenes* and to counter resistance development by this organism on RTE processed meat products.

Therefore, the objective of this study was to assess the initial inhibitory effectiveness of combinations of pediocin (in ALTA™ 2341) and post-packaging irradiation on *L. monocytogenes* on frankfurters in different package types (single-link, 5-link and 10-link packages). Subsequently, samples treated with selected treatment combinations were stored at 4°C, 10°C and 25°C for up to 12 weeks to assess inhibitory effectiveness during storage. In addition, this study assessed changes in the physical and chemical quality, including sensory quality, of frankfurters subjected to selected antimicrobial treatments.
Materials and Methods

The experimental protocol was designed to first compare the effects of pediocin concentrations and post-packaging irradiation pasteurization for immediate reduction of *L. monocytogenes* on frankfurters in different package types. The most effective combinations were then evaluated for inhibitory effects during storage at 4°C, 10°C and 25°C. Finally, the most effective treatments were assessed for product quality changes.

Frankfurter preparation

Frankfurters were manufactured from frozen beef trim (~80% lean) and fresh pork trim (~50% lean) purchased from commercial suppliers. Frozen beef trim were removed from the freezer (-20°C) 24 h before processing and tempered at 2°C to 4°C. All trimmings were coarse ground (0.95-cm plate) and fat content measured by Anyl-Ray (Kartridg-Pak Co., Inc., Davenport, IA). Frankfurters were formulated with a 45.4 kg meat block, containing 22.7 kg of beef trim and 22.7 kg of pork trim, plus 9.1 kg of ice/water, 1.49 kg of spice mixture (A.C. Legg Packing Co., Inc., Birmingham, AL), 112 g of curing salt (A.C. Legg Packing Co., Inc., Birmingham, AL) and 908 g of salt.

The lean beef trim was chopped with ice/water, spice and cure to about 4.5°C after which the fat pork trim was added and chopping continued to 13.9°C to form the batter. The frankfurter batter was vacuum-stuffed (Risco Model RS 4003-165; Stoughton, MA) into 24 mm cellulose casings and linked at 12 to 14 cm in length. Stuffed frankfurters were smoked, cooked and showered in an Alkar oven (Alkar, Lodi, WI). Chilling was achieved in a 2°C cooler overnight before peeling. Finished, peeled frankfurters were stored at 2°C for 12 to 18 h before random assignment to different experimental treatments. Finished weight of the frankfurters was 45 ± 5 g per link.

Fat, moisture (1) and protein content (2) of finished products were measured with the results indicating the frankfurters contained 28.25% crude fat, 53.66% moisture and 13.44% protein.

Inoculation and packaging

Frankfurters were packaged in vacuum bags using three package types as follows: 1) 10 links per package in a double row of 5 each, 2) 5 links per package in a single row, and 3) singly packaged links. Each packaging group was divided into two subgroups for addition of one of two levels of pediocin (ALTA™ 2341, Quest Int., Sarasota, FL) before inoculation with *L. monocytogenes*. ALTA™ 2341 was formulated at 40% (wt/vol) in sterile, distilled water to achieve 3,000 arbitrary units (AU) of pediocin per ml. The frankfurters were placed in bags, then sprayed with either 1 or 2 ml per link of 40% ALTA™ 2341 suspension, equivalent to 3,000 AU or 6,000 AU pediocin per link (Pdn-3000 or Pdn-6000). The vacuum bags (Cryovac® B-2540, Cryovac Sealed Air Corp., Duncan, SC, water vapor transmission = 0.5-0.6 g at 100°F, 100% RH/100 sq. in./24 h; oxygen transmission rate = 3-6 CC at 40°F/m²/24 h/0% RH), with frankfurters and pediocin were hand-massaged for 5 s to 10 s for even distribution of pediocin, before vacuum-sealing (Multivac A 300/52, Mutivav Sepp Haggenmuller GmbH & Co., Wolfertschwenden, Germany).
For inoculation of the frankfurters, a 5-strain cocktail mixture of \textit{L. monocytogenes} cultures including \textit{L. monocytogenes} Scott A, H7764 1/2a, H7969 4b, H7962 4b and H7762 4b, was used. With exception of the Scott A strain, all strains were obtained from the CDC as clinical isolates from the Bil Mar Foods outbreak of 1998-1999 (CDC, Atlanta, GA). The \textit{L. monocytogenes} cultures were individually grown in trypticase soy broth containing 0.6% yeast extract (TSB-YE broth) (Difco, Becton Dickinson and Company, Sparks, MD) at 35°C for 24 h. Then, 1 ml of culture from each individual strain was combined to give a 5-ml mixed culture of \textit{L. monocytogenes}. The mixed culture was transferred to 500-ml TSB-YE broth and incubated at 35°C for 24 h to reach the stationary phase. The final concentration of the original 5-strain mixture of \textit{L. monocytogenes} was 9.30 log CFU/ml.

Inoculation of samples was done by adding 1 ml per link of the original mixture of \textit{L. monocytogenes} (9.30 log CFU/ml) to result in a “high” inoculation level. The original mixture was then diluted 1:100 (7.30 log CFU/ml) to result in a “low” inoculum when 1 ml per link was used. The inoculum was added to the frankfurters after they were placed in the appropriate package and before the package was sealed. Inoculated frankfurters without addition of pediocin served as controls and were used to determine the recovery of \textit{L. monocytogenes}.

After inoculation, the packages were hand-massaged 5 s to 10 s to evenly distribute the 5-strain mixture of \textit{L. monocytogenes} on the surface of each frankfurter, and closed under vacuum. After vacuum-packaging, all frankfurters were placed in cardboard boxes and stored at 2°C to 4°C for 14-18 h before sampling. Average recovery of \textit{L. monocytogenes} from untreated samples was 3.40 and 5.20 log CFU/g for the low and high inoculation levels, respectively.

\textbf{Irradiation treatments}

For post-packaging irradiation pasteurization, inoculated samples were treated with 1 kGy or 2 kGy of irradiation doses using single or double-side irradiation treatments at the Linear Accelerator Facility (Linear Accelerator-Circle III R, Saint-Aubin, France) at Iowa State University. The actual measured average of absorbed irradiation doses (minimum and maximum doses) was 1.20 (0.93-1.71) and 2.30 (1.82-3.37) kGy for single-pass treatments and 1.40 (1.29-1.53) and 3.50 (2.54-6.40) kGy for double-pass treatments, respectively. The double-pass treatment was necessary for the double-row packages of frankfurters because of the package thickness.

Actual absorbed doses were measured by placing alanine pellets on both sides of a package. An electron paramagnetic resonance (EPR) instrument was used to determine the absorbed dose (Bruker Instruments, Inc., Billerica, MA). The instrument was calibrated with standard dosimeters provided by the National Institute of Standards and Technology (NIST). Reported doses were averages from absorbed doses obtained at the top surface and bottom surface of the samples.
Initial evaluation of treatments

Survival of *L. monocytogenes* that was surface-inoculated (3.40 or 5.20 log CFU/g) on frankfurters was determined by enumerating the surviving organisms. The effectiveness of the treatment combinations for reducing *L. monocytogenes* counts was compared to determine which were the most effective combinations for subsequent study during refrigerated storage.

Evaluations during storage

Based on initial evaluation of the treatments, those treatments that showed the greatest immediate inhibitory effectiveness were selected for subsequent evaluation of long-term effectiveness during storage. Treatments that were selected included both levels of ALTA™ 2341 (Pdn-3000 or Pdn-6000) in combination with post-packaging irradiation pasteurization at 1.2, 2.3 or 3.5 kGy. Untreated samples, surface-inoculated with 3.40 log CFU/g, served as control samples and were checked for recovery. The treatment combinations of pediocin with irradiation also included package types as follows: 1-link/Pdn-6000/2.3 kGy, 5-link/Pdn-3000/2.3 kGy, 5-link/Pdn-6000/1.2 kGy, 5-link/Pdn-6000/2.3 kGy, and 10-link/Pdn-6000/3.5 kGy. Data from samples treated with pediocin alone (9) was used for comparisons to the five selected treatments. The single-link or 5-link package types were compared for inhibitory effectiveness of *L. monocytogenes* using the Pdn-6000/2.3 kGy treatment. Inoculation used in all samples for the evaluations during storage were ca. 3.40 log CFU/g of the same 5-strain cocktail mixture of *L. monocytogenes* used for the initial evaluation. Storage of samples was at 4°C, 10°C and 25°C for up to 12 weeks.

Microbiological analyses

Initial evaluation of treatments. Packages of frankfurters were aseptically opened, using sterile scissors, 18 h after treatments. Except for single-link packages, sampling was done by collecting two frankfurters, one from the center of the package row and one from the outside. For 10-link packages, the frankfurters were taken from different rows. Frankfurters were aseptically cut in half (20-23 g portions) with sterile scissors and tweezers. Samples were homogenized (Seward Stomacher blender, Model 4000, Tekmar™ Co., Cincinnati, OH) for two min in sterile stomacher bags (Whirl-Pak Filter Bag B01318, A Nasco, Ft. Atkinson, WI) with sufficient 0.1% sterile peptone water to give a 1:5 dilution of the sample. *L. monocytogenes* cells were enumerated by serially diluting 1 ml of the blended sample in 9 ml of 0.1% peptone water, plating on Modified Oxford (MOX) (Difco) agar plus 0.1% Oxford Antimicrobial Supplement. Samples were also plated on TSA plus 0.6% yeast extract (TSA-YE) (Difco). Incubation of all plates was at 35°C for 48 h. Typical colonies of *L. monocytogenes* were enumerated, identified by gram stain and confirmed using API *Listeria* kits (bioMerieux, Inc., Hazelwood, MO).

Evaluations during storage. For enumeration of samples held in storage, packages were opened aseptically and whole frankfurters were mixed for 1 min in a Seward stomacher blender with an equal amount of 0.1% peptone water (50% dilution) to rinse them thoroughly. *L. monocytogenes* cells were enumerated by serial dilution using 1 ml of the rinse in 9 ml of 0.1% peptone water, plating on MOX agar and TSA-YE, with
identification as described previously. When increased sensitivity was required, 1.0 ml samples of rinse were plated directly onto MOX or TSA-YE.

**Product quality analyses**

After completion of the microbiological evaluations, the treatments that had the most impact on *L. monocytogenes* were also evaluated for product quality changes. Frankfurters (5 links per package) were prepared, processed and treated in the same way as for the inoculation challenge, but without the *L. monocytogenes* inoculation. Quality evaluation included measurement of purge accumulation, color, texture, odor quality, pH and thiobarbituric acid (TBA) values.

**Physical and chemical analyses of frankfurters**

For purge accumulation, two packages were each weighed, opened and the frankfurters removed. The packages and links were wiped dry and reweighed. The weight difference was calculated as purge and expressed as percentage of unpackaged product weight (6).

Color (L*, a*, b*) measurements utilized a Hunter Labscan spectrophotometer (Hunter Associated Laboratories, Inc., Reston, VA), using illuminant A and 10° observer (incandescent light) with a 0.25 in. port insert. Samples were overwrapped with clear saran film and surface color was measured at two locations (center and end) of each frankfurter. Five links from each treatment were measured for color. For texture measurement, a Texture Analyzer (Model TA.XT 2 i, Godalming, U.K.) was used for assessment of skin toughness and interior firmness by measuring surface puncture resistance and interior textural firmness with a 3-mm puncture probe. Five frankfurters from each treatment were measured in the center and the end of each link. The probe was programmed to penetrate 12 mm into the samples following measurement of the surface skin resistance. Penetration speed was 1.5 mm/second. All samples were measured at room temperature 3 h after removal from the refrigerator.

For pH measurement, 10 g of sample was blended with 90 ml of distilled water in a Waring Blender® and the slurry measured with a pH meter (Fisher Accumet Model 925, Fisher Scientific, Pittsburgh, PA) using a sealed combination electrode (Omega Engineering, Inc., Stamford, CT). The TBA values were measured using the modified method for cured meats (34). Duplicate measurements of pH and TBA values were recorded for each sample.

**Sensory evaluation**

The sensory evaluation was conducted using a panel of 16 trained panelists, all being students, staff or faculty in the Department of Food Science and Human Nutrition at Iowa State University. All panelists were volunteers and were trained using commercial frankfurters, for the odor notes and terminology to be measured, and the scales used. Only uninoculated samples were used for sensory evaluation. Panelists evaluated samples for purge, color, texture, and odor using 15-cm unstructured line scale. Fluorescent-lighted booths were used for sample presentation to the panelists.

The amount of purge and exterior color was scored using intact unopened packages of frankfurters. For the texture assessment, the panelists used the edge of a dinner fork to cut a cross-section thorough the center
of a frankfurter. For odor evaluation, frankfurters were heated in boiling water for 2 min, cut into sections and placed in 150-ml covered containers before presentation to the panel. Panelists evaluated odor for smoky, burnt and acidic traits; all of which were established during the training session. The numerical scales used for sensory intensity of purge, color, texture and odor were described as; 0 = none, extremely light, extremely soft or none, respectively; while 15 = extremely abundant, dark, firm or intense, respectively. All sensory evaluations were conducted within two weeks of processing and were repeated three times.

**Statistical analyses**

Microbiological data were transformed into logarithms of the number of colony-forming units (log_{10} CFU/g). The Statistical Analysis System (27) was used to determine means, standard errors and variance analyses from the three replications. When analysis of variance (ANOVA) revealed a significant difference (P < 0.05), treatment means were compared using the least significant difference (LSD) test.

**Initial evaluation of treatments.** Data from the initial evaluation were treated as a split, split-plot design with *L. monocytogenes* inoculation level and packaging types as the main plot, pediocin concentration as the split plot, and irradiation doses as the split-split plot. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

**Evaluations during storage.** The data from the evaluations during storage was analyzed as a split plot with 8 treatments in the main plot while the subplot consisted of 11-13 sampling dates. The sampling dates were either day 0 to day 10 or week 0 to week 12 depending on storage temperature. All data were analyzed using SAS with the general linear model (GLM) procedure. Comparisons of means were based on Tukey’s range test for least significant differences.

**Results**

**Initial evaluation of treatments**

**Effectiveness of irradiation doses.** *L. monocytogenes* was not detected on MOX plates and very few aerobic bacteria (A.P.C.) were detected on TSA-YE plates from uninoculated frankfurters. Survival of *L. monocytogenes* on inoculated frankfurters, treated with irradiation or with pediocin and irradiation, is shown in Fig. 1. *L. monocytogenes* populations were reduced by post-packaging irradiation alone without pediocin (Fig. 1-A). For frankfurters surfaced-inoculated with the lower initial inoculation level of *L mocytogenes* (3.40 log CFU/g), *L monocytogenes* populations were reduced to undetectable levels by irradiation (single-link and 5-link packages) with 2.3 kGy or greater, or by the double-pass irradiation (10-link packages) with 1.4 kGy or greater. The reduction achieved by the 1.2 kGy dose was 2.3 to 2.4 logs, regardless of package types. Frankfurters with the higher initial level (5.20 log CFU/g) of *L monocytogenes* showed more survivors. Samples in 10-link packages irradiated at 3.5 kGy (double-pass irradiation) showed the initial populations of 5.20 log CFU/g to be reduced to undetectable levels. The double-pass irradiation of 10-link packages with 1.4 kGy achieved a 3.7-log reduction of *L monocytogenes* from the high inoculum level.
Figure 1. Survival of *Listeria monocytogenes* and aerobic microorganisms on the surface of frankfurters treated with pediocin (in ALTA™ 2341) and post-packaging irradiation; A. *L. monocytogenes* counts (3.40 & 5.20 log CFU/g inoculations) on MOX agar, B. Aerobic bacterial counts (A.P.C.) on TSA-YE agar.

Irrad.: Frankfurters (1, 5 and 10 link[s]/pkg) irradiated at 1.2, 1.4, 2.3 and 3.5 kGy
Irrad.-Pdn-3000: Frankfurters (1, 5 and 10 link[s]/pkg) treated with 3,000 AU pediocin and irradiated at 1.2, 1.4, 2.3 and 3.5 kGy
Irrad.-Pdn-6000: Frankfurters (1, 5 and 10 link[s]/pkg) treated with 6,000 AU pediocin and irradiated at 1.2, 1.4, 2.3 and 3.5 kGy
0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
Irradiation treatments alone dramatically reduced *L. monocytogenes* populations on frankfurters. Effects of irradiation alone achieved from 2.3 to 3.4-log and 1.7 to 5.2-log reductions for 3.40 and 5.20 log CFU/g inoculations, respectively. In general, effects of irradiation were greater than effects of pediocin alone which achieved from 1.5 or 1.8-log (Pdn-3000) and 1.6 or 2.1-log (Pdn-6000) reductions. The only exception was the frankfurters in 5-link packages inoculated with 5.20 log CFU/g and irradiated at 1.2 kGy. This irradiation treatment resulted in a 1.7-log reduction which was less than that for samples treated with Pdn-6000 alone.

**Effects of package types.** The surviving populations of the pathogen on samples in single-link packages were similar to samples in 5-link packages (Fig. 1-A). The survival in single-link and 5-link packages for the 3.40 log CFU/g inoculation were 1.13 and 1.05 log CFU/g for samples irradiated with 1.2 kGy dose. Counts were reduced to undetectable levels on similar samples irradiated with 2.3 kGy dose. For the 5.20 log CFU/g inoculation reductions, the surviving populations were 2.81 and 3.47 log CFU/g on samples in single-link and 5-link packages respectively, irradiated with 1.2 kGy dose. Survivors were measured at 1.14 and 1.56 log CFU/g on similar samples irradiated with the 2.3 kGy dose.

Generally, the higher doses of irradiation resulted in less pathogen survival; and decreased the pathogen populations in smaller package types.

**Antilisterial effects of treatment combinations**

**Pediocin and irradiation.** In Fig. 1-A, the results show that when irradiation at 1.2 kGy or greater and pediocin at 3,000 AU (Pdn-3000) or greater were combined, a 50% or more reduction of initially inoculated *L. monocytogenes* numbers was achieved. Similar results required 2.3 kGy or more of irradiation if used alone. A complete reduction of the population was achieved in samples irradiated at 1.2 kGy when Pdn-3000 was included for the 3.40 log CFU/g inoculation. Irradiation at 2.3 kGy combined with pediocin at 6,000 AU (Pdn-6000) achieved reduction to undetectable levels for the 5.20 log CFU/g inoculation. Similar effects required 3.5 kGy at this inoculation level for irradiation alone. Addition of pediocin (Pdn-3000 or Pdn-6000) reduced counts on samples inoculated with 3.40 log CFU/g by 1.5 to 1.8 logs, and including a 1.2 kGy or greater irradiation dose resulted in further reduction of counts to undetectable levels (a 3.40-log reduction).

For the higher initial inoculation (5.20 log CFU/g), samples with pediocin (Pdn-3000 or Pdn-6000) showed 1.6 to 2.1-log reductions. Combining irradiation at 1.2 kGy with pediocin at either level, resulted in further reductions of 3.1 or 4.0 logs. Irradiation with 2.3 kGy and Pdn-3000 reduced this pathogen to very low levels (0.06 to 0.19 log CFU/g), reaching ca. 5.0 or 5.1-log reduction in numbers.

The irradiation doses at 1.2 kGy, even combined with pediocin, were insufficient to reduce the higher inoculation of *L. monocytogenes* (5.20 log CFU/g) to undetectable levels. In contrast, for the lower inoculation (3.40 log CFU/g), the pathogen populations were reduced to undetectable levels by 1.2 kGy doses.

**Effects of package types.** Figure 1-A shows that package types did not affect antilisterial effectiveness of the treatment combinations. There was no difference in reduction of pathogen numbers
between single-link and 5-link packages treated with pediocin and irradiation. The 10-link packages differ
because these packages received greater total irradiation doses than the single-link or 5-link packages.

**Synergistic effects of pediocin and irradiation.** The data in Fig.1-A show that the treatment
combinations of pediocin and irradiation effectively reduce the populations of *L. monocytogenes* on frankfurters
after treatments, and suggest a synergistic effect by most combinations of these two factors. The population
reduction of this pathogen was increased by both higher levels of pediocin and higher doses of irradiation. It
appears that post-packaging irradiation pasteurization achieved a significant pasteurization effect which was
dose-dependent and which was potentiated by the presence of pediocin. There was a significant (P < 0.001)
interaction between irradiation and addition of pediocin. The presence of pediocin appears to result in a
synergistic effect on *L. monocytogenes* when combined with irradiation because the interaction was very
significant (P < 0.0001).

**The interactions among treatments for *L. monocytogenes* inactivation.** According to the results
from statistical analyses, there were significant (P< 0.0001) interactions between initial inoculation numbers of
*L. monocytogenes*, pediocin concentrations, absorbed doses of irradiation and package types. The initial
inoculation numbers, absorbed doses and package types also showed a 3-way interaction (P < 0.0001) between
these three factors. This means that the effectiveness of the treatment combinations was significantly (P <
0.0001) affected by the initial inoculation levels.

**Comparison for recovery of *L. monocytogenes* on MOX agar and TSA-YE.** The data for aerobic
bacterial populations (A.P.C.) in Fig. 1-B (TSA-YE) supported the results shown in Fig. 1-A (MOX).
Comparison of the recovery from the different agars demonstrated that the results from TSA-YE were greater
than that from MOX agar. The use of TSA-YE includes other organisms in addition to *L. monocytogenes* but
also may permit better recovery of injured *L. monocytogenes* cells. In all cases there were similar patterns for
the survival populations. It was evident that the colonies grown on TSA-YE were predominantly *L.
monocytogenes* due to colonies that had uniform appearance, and that were typical of *Listeria* spp.

**Evaluations during storage**

From the results of the initial evaluations, the most effective treatments were selected for storage tests.
The selected treatments included irradiation at 1.2, 2.3 or 3.5 kGy combined with pediocin in all three package
types (single-link, 5-link or 10-link packages). The packages were stored at 4°C, 10°C and 25°C. The
treatments that were irradiated at 2.3 kGy included both concentrations of pediocin, but those treatments
irradiated at 1.2 or 3.5 kGy included only the greater pediocin level (Pdn-6000) because antilisterial effects
were greater than with Pdn-3000. The objective of this part of the study was to compare the antilisterial
effectiveness of these treatments during storage of frankfurters at 4°C, 10°C and 25°C. All of the frankfurters
used for the storage studies were inoculated with *L. monocytogenes* at 3.40 log CFU/g.

**Survival and growth of *L. monocytogenes* on treated frankfurters during storage at 4°C**

**Comparison of growth curves among treatments.** The data in Fig. 2 shows the surviving
populations of *L. monocytogenes* (3.40 log CFU/g inoculation) on treated frankfurters during storage at 4°C. In
Figure 2. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging irradiation during storage at 4°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE agar) C. Treatments for frankfurters treated with Pdn-3000 or Pdn-6000 and irradiated at 1.2, 2.3 or 3.5 kGy in 5-link or 10-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in single-link, 5-link and 10-link packages treated with Pdn-6000 and irradiated at 2.3 or 3.5 kGy (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
06-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-P1-3000-1.2: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin and irradiated at 1.2 kGy
05-P1-3000-2.3: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin and irradiated at 2.3 kGy
10-P1-3000-3.5: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and irradiated at 3.5 kGy
05-P1-6000-2.3: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 2.3 kGy
05-P1-6000-3.5: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 3.5 kGy
01-P1-6000-1.2: Frankfurters (1 link / pkg) treated with 6,000 AU pediocin and irradiated at 1.2 kGy

0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
Fig. 2-A, the treatments with pediocin alone, at either level, reduced the initial population of *L. monocytogenes* (9). The antilisterial effectiveness of the pediocin was clearly enhanced by combination with irradiation treatments and it was greater than that of pediocin in combination with thermal pasteurization (9). The combination of pediocin and irradiation had very marked effects when samples were stored at 4°C (Fig. 2-A). All combination treatments resulted in virtually undetectable levels (*P* > 0.05) of *L. monocytogenes* for up to 12 weeks except for the 5-link/Pdn-6000/1.2 kGy combination. However, the counts of this treatment were slow to increase. Recovery of the pathogen from control samples was 3.47 log CFU/g at week 0. At the first 7 to 8 weeks, the population of the pathogen remained relatively constant at 3.17 to 3.82 log CFU/g at 4°C. After storage for 7 to 8 weeks, the surviving populations of the pathogen began growing, increasing to 4.78 log CFU/g (*P* < 0.05) at week 12.

**Antilisterial effectiveness of irradiation doses and pediocin concentrations.** Figure 2-C shows results from 5-link packages treated with irradiation at 1.2 or 3.5 kGy and Pdn-6000 only, and packages treated at 2.3 kGy with both pediocin concentrations. These combined treatments were more (*P* < 0.05) effective than pediocin alone (Fig. 2-A) during storage at 4°C. The growth curves for the populations following these four treatment combinations did not differ (*P* > 0.05) from one another. At week 0, the selected treatment combinations provided immediate reduction of the pathogen populations, to levels of 0.00 to 0.51 log CFU/g, compared to control samples (3.47 log CFU/g). During storage for 12 weeks, the selected treatments resulted in lower survival (*P* < 0.05) compared to the control. The 5-link/Pdn-6000/1.2 kGy treatment showed recovery and growth of *L. monocytogenes* after 7 weeks. However, the increasing numbers were not significantly (*P* > 0.05) higher than the other irradiation treatments for the rest of the storage period.

In general, the populations of the pathogen on samples treated with Pdn-3000 or greater and irradiated with 2.3 kGy remained undetectable throughout storage for 12 weeks at 4°C. These results imply listericidal effects for these treatments. The irradiation doses of 2.3 kGy or greater were significantly (*P* < 0.05) more effective than 1.2 kGy. However, there was no significant (*P* > 0.05) difference between the effectiveness of the pediocin concentrations with the 2.3 kGy irradiation dose.

**Effects of package types on *L. monocytogenes***. Comparison of the results for the different package types (single-link and 5-link packages) with Pdn-6000, and irradiated with 2.3 kGy, are shown in Fig. 2-D. The 10-link packages were not included in this comparison because of the higher absorbed doses resulting from double-pass irradiation. There were no significant (*P* < 0.05) differences between single-link and 5-link packages and both showed little or no growth during the 12-week of storage period. After the treatments at week 0, these two package types provided an immediate reduction of *L. monocytogenes* counts (0.30 to 0.51 log CFU/g). The results showed that the interfacial areas between links in the 5-link packages did not affect the antilisterial impact of irradiation as was the case for thermal pasteurization (10).

The surviving aerobic bacterial populations counted on TSA-YE (Fig. 2-B) were primarily *L. monocytogenes* and were similar to, though slightly higher than, counts on MOX agar (Fig. 2-A) during storage for 12 weeks. It was observed that the treatments that inhibited or slowed growth of *L. monocytogenes* as
determined on MOX gave the same results using TSA-YE, a medium that enhances recovery of the pathogen because it lacks the selective antimicrobials included with MOX agar. While TSA-YE includes other organisms in addition to *L. monocytogenes*, it is evident that most of the recovered organisms on TSA-YE were *L. monocytogenes*.

**Survival and growth of *L. monocytogenes* on treated frankfurters during storage at 10°C**

**Comparison of growth curves among treatments.** The data in Fig. 3 show the antilisterial effectiveness of combinations of pediocin with irradiation during storage at 10°C. The *L. monocytogenes* populations on the control increased immediately after week 0. The combination treatments fall into two categories similar to results at 4°C. All selected treatments had significantly (P < 0.05) lower recoveries than the control or treatments with pediocin alone. The only exception was 5-link/Pdn-6000/1.2 kGy combination which recovered more quickly after about 1 to 2 weeks of storage (Fig. 3-A). Again, the effect of irradiation pasteurization was greater than that of thermal pasteurization and interactive effects between pediocin and irradiation occurred. Pediocin alone reduced *L. monocytogenes* counts initially. But the combinations of pediocin and irradiation achieved greater initial reduction of *L. monocytogenes* counts. After delayed growth of 3 to 12 weeks, most of the treated samples showed erratic recovery with reduced population numbers, except for the 5-link/Pdn-6000/1.2 kGy treatment. The 5-link/Pdn-6000/1.2 kGy treatment showed consistent growth after a lag phase of 2 weeks. The other four selected treatments suppressed growth throughout storage for 12 weeks at 10°C. Recoveries were erratic suggesting extensive damage to cells by irradiation.

**Antilisterial effectiveness of irradiation doses and pediocin concentrations.** Figure 3-C shows changes in the pathogen populations resulting from treatment combinations of irradiation and pediocin for 5-link packages and for 10-link packages (double-pass irradiation) treated with 3.5 kGy relative to controls. At week 0, the populations of the pathogen following these 4 selected treatments was reduced significantly (P < 0.05), to essentially undetectable levels compared to initial populations of 3.38 log CFU/g on untreated control samples. The population of the pathogen on control samples showed an immediate increase in numbers after 1 week of storage. After storage for 6 weeks, *L. monocytogenes* populations increased significantly (P < 0.05) to 5.70 log CFU/g and achieved maximum numbers (5.77 log CFU/g) after 9 weeks of storage. The surviving population of the pathogen on samples from the 5-link/Pdn-6000/1.2 kGy combination increased relatively quickly after a lag phase of 2 weeks and grew more rapidly (P < 0.05) than the others to 4.01 log CFU/g after 4 weeks. The comparable treatment with Pdn-3000 instead of Pdn-6000 did not show consistent growth for 3.5 to 5 weeks. No substantial (P > 0.05) changes in the counts of the pathogen occurred on samples from the 10-link/Pdn-6000 /3.5 kGy combination. The population of these samples was mostly undetectable and did not exceed 0.58 log CFU/g throughout storage for 12 weeks. The absorbed dose of irradiation at 3.5 kGy combined with Pdn-6000 appeared to result in listericidal effects. Again, it is evident that the antilisterial effectiveness of absorbed doses of irradiation was greater (P < 0.05) than that of pediocin concentrations. There was a synergistic effect between pediocin and irradiation at 10°C similar to 4°C. However, there was no significant (P > 0.05) difference between the pediocin concentrations at the 2.3 kGy

Figure 3. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging irradiation during storage at 10°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE agar) C. Treatments for frankfurters treated with Pdn-3000 or Pdn-6000 and irradiated at 1.2, 2.3 or 3.5 kGy in 5-link or 10-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in single-link, 5-link and 10-link packages treated with Pdn-6000 and irradiated at 2.3 or 3.5 kGy (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
05-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-Pdn-6000-1.2: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 1.2 kGy
05-Pdn-3000-1.2: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin and irradiated at 1.2 kGy
10-Pdn-6000-3.5: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and irradiated at 3.5 kGy
05-Pdn-6000-3.5: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 3.5 kGy
01-Pdn-6000-2.3: Frankfurters (3 link / pkg) treated with 6,000 AU pediocin and irradiated at 2.3 kGy
01-Pdn-6000-1.2: Frankfurters (3 link / pkg) treated with 6,000 AU pediocin and irradiated at 1.2 kGy

0.00 log CFU/g represents an undetectable number of *L. monocytogenes*
irradiation dose when all package were considered.

**Effects of package types on *L. monocytogenes***. Figure 3-D compares the antilisterial effects of pediocin at 6,000 AU (Pdn-6000) and 2.3 kGy irradiation dose in different package types during storage at 10°C. No significant (P > 0.05) increase in the pathogen populations occurred on any samples during storage for 12 weeks.

The results of storage at 10°C showed that the pathogen populations were reduced by addition of pediocin and accumulated irradiation doses. As shown in Figure 3-A, all samples treated with Pdn-3000 and irradiated at 2.3 kGy or greater showed limited *L. monocytogenes* growth and were not significantly (P > 0.05) different from one another. The antilisterial effectiveness of the 10-link/Pdn-6000/3.5 kGy treatment did not differ greatly (P > 0.05) from the single and 5-link packages despite the higher absorbed dose (double-pass irradiation).

The growth of aerobic bacterial populations (A.P.C.) on TSA-YE (Fig. 3-B) was similar to that on MOX agar (Fig. 3-A), though the counts on TSA-YE were slightly higher than those on MOX agar. The results at 10°C were consistent with the growth patterns observed for samples stored at 4°C.

**Survival and growth of *L. monocytogenes* on treated frankfurters during storage at 25°C**

**Comparison of growth curves among treatments.** In Fig. 4, the effects of combining pediocin and irradiation treatments are evident even with subsequent storage at 25°C. In Fig. 4-A, pediocin appears to delay *L. monocytogenes* growth but there was no significant (P > 0.05) difference between pediocin-treated samples, which were intermediate in effectiveness or between the pediocin treatments and the control. However, the combined treatments are significantly (P < 0.05) different from both pediocin-only samples and controls, and provided the greatest antilisterial effectiveness. The results clearly showed the antilisterial effectiveness of combinations of irradiation and pediocin. After 1 day of storage, the pathogen population on control samples increased (P < 0.05) to reach 4.85 log CFU/g and the numbers achieved the maximum levels of 5.85 log CFU/g after only 3 days at 25°C.

**Antilisterial effectiveness of irradiation doses and pediocin concentrations.** Figure 4-C shows the antilisterial effectiveness of the selected treatment combinations (Pdn-6000/1.2 kGy, Pdn-3000/2.3 kGy and Pdn-6000/2.3 kGy in 5-link packages and Pdn-6000/3.5 kGy in 10-link packages [double-pass irradiation]). There was a short delay in growth of the pathogen during the first 2 to 3 days on the samples in 5-link packages. After the first 2 to 3 days, there was no significant (P > 0.05) difference among the three 5-link package treatments. The pathogen numbers showed erratic recoveries probably due to the wide range of absorbed doses of irradiation and damage to the cells. The pathogen numbers from the three 5-link package treatments did not show significant (P > 0.05) growth and did not exceed 4.09 log CFU/g. No marked changes in the population from the 10-link/Pdn-6000/3.5 kGy treatment occurred for up to 12 days, probably due to the relatively high absorbed dose of irradiation (3.5 kGy). The results showed that the higher irradiation doses with pediocin achieved greater reduction of *L. monocytogenes* numbers.

**Effects of package types on *L. monocytogenes***. Figure 4-D compares the antilisterial effectiveness of
Figure 4. Survival and growth of *Listeria monocytogenes* (3.40 log CFU/g inoculation) and aerobic bacteria on the surface of frankfurters treated with pediocin (in ALTA™ 2341) combined with post-packaging irradiation during storage at 25°C; A. All selected treatments listed below (*L. monocytogenes* on MOX agar), B. All selected treatments listed below (aerobic bacteria [A.P.C.] on TSA-YE agar) C. Treatments for frankfurters treated with Pdn-3000 or Pdn-6000 and irradiated at 1.2, 2.3 or 3.5 kGy in 5-link or 10-link packages (*L. monocytogenes* on MOX agar), D. Frankfurters in single-link, 5-link and 10-link packages treated with Pdn-6000 and irradiated at 2.3 or 3.5 kGy (*L. monocytogenes* on MOX agar)

05-Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
06-Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
05-P1-3000-1.2: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin and irradiated at 1.2 kGy
05-P1-6000-1.2: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 1.2 kGy
10-P1-6000-3.5: Frankfurters (10 links / pkg) treated with 6,000 AU pediocin and irradiated at 3.5 kGy
05-P1-6000-2.3: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated at 2.3 kGy
01-P1-6000-2.3: Frankfurters (1 link / pkg) treated with 6,000 AU pediocin and irradiated at 2.3 kGy
6.00 log CFU/g represents an undetectable number of *L. monocytogenes*
Pdn-6000 and 2.3 kGy irradiation in different package types. The single link package treatment slightly delayed growth of the pathogen for about 3 to 5 days and was not significantly (P > 0.05) different from that for samples in 5-link packages. Because of the higher absorbed dose of irradiation, the inhibitory effectiveness for the 10-link/Pdn-6000/3.5 kGy treatment was greater compared to samples in 5-link packages. It appears that Pdn-6000 combined with irradiation at 2.3 kGy or greater is necessary to inhibit the growth of *L. monocytogenes* on frankfurters stored at 25°C. The irradiation dose of 3.5 kGy provided complete inhibition of growth of the pathogen.

It is evident that the aerobic bacterial populations (A.P.C.) recovered from TSA-YE (Fig. 4-B) were greater than those from MOX agar (Fig. 4-A). In this case, the shapes of most of the colonies were not typical of *L. monocytogenes* and were accompanied by a spoilage aroma, especially during the last few days of storage. It is well-known that *L. monocytogenes* is not a good growth competitor compared to normal spoilage bacteria. The surviving spoilage organisms easily dominated in packages when samples were stored at 25°C. However, the 10-link/Pdn-6000/3.5kGy treatment was effective enough to control aerobic bacterial cells and kept them at levels below 3.10 log CFU/g.

**Effects of storage temperatures.** Growth of surviving populations of *L. monocytogenes* on frankfurters was the most rapid at 25°C (Fig. 4-A) compared to 4°C (Fig. 2-A) and 10°C (Fig. 3-A) as expected. The population reduction on samples during the first 1 to 2 weeks at 10°C was similar to those for the same treatments stored at 4°C. After about 2 weeks, the pathogen populations on samples stored at 10°C grew faster than those for the same treatments stored at 4°C. Irradiation combined with pediocin was effective at all three storage temperatures. Irradiation appeared to be synergistic with the pediocin treatments at 4°C and 10°C. The 2.3 kGy dose combined with pediocin was sufficient to reduce *L. monocytogenes* to undetectable levels for 12 weeks.

**Product Quality Analyses**

Based on the microbiological results, the most effective combinations were evaluated for potential product quality changes that might be introduced by the treatments. The treatments selected for sensory evaluation included irradiation at two doses (1.2 kGy and 2.3 kGy) with 6,000 AU of pediocin (Pdn-6000).

**Effects of selected treatments on physical and chemical attributes.** The results for purge, color, texture, pH and oxidative change (TBA values) are presented in Table 1. There was no effect of treatments on package purge, except for the volume of solution added to packages. The addition of Pdn-6000, for example, resulted in a significant (P < 0.05) increase in the water measured as purge. Irradiation treatments, however, did not alter the purge accumulation in the packages.

In Table 1, The irradiation treatments resulted in color which was darker (L* value) and redder (a* value) than controls. These differences were not large but were statistically significant (P < 0.05) and were confirmed by sensory panel evaluations. Textural characteristics were not affected by any of the product treatments, either for exterior skin toughness or for interior firmness. Product TBA values were also unaffected by the treatments, but pH values were significantly (P < 0.05) different compared to the control.
Table 1. Comparison of physical and chemical analyses of frankfurters following selected treatments with pediocin (in ALTA™ 2341) and post-packaging irradiation

<table>
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<tr>
<th>Treatments</th>
<th>Purge (%)</th>
<th>Color</th>
<th>Firmness (kg)</th>
<th>pH</th>
<th>TBA (mg MDA /kg)</th>
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<td></td>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
<td>Skin</td>
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<tr>
<td>Control</td>
<td>0.61 ± 0.25</td>
<td>44.98 ± 0.90</td>
<td>15.14 ± 1.12</td>
<td>13.67 ± 0.90</td>
<td>0.40 ± 0.03</td>
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<tr>
<td></td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>05-Pdn-3000¹</td>
<td>3.33 ± 0.62</td>
<td>44.38 ± 1.50</td>
<td>15.76 ± 0.97</td>
<td>13.35 ± 0.71</td>
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<tr>
<td></td>
<td>(b)</td>
<td>(ab)</td>
<td>(ab)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>05-Pdn-6000²</td>
<td>5.13 ± 0.67</td>
<td>42.93 ± 1.03</td>
<td>15.93 ± 1.41</td>
<td>13.19 ± 0.49</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>(bc)</td>
<td>(bc)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>05-PI-6000-1.2³</td>
<td>5.38 ± 1.12</td>
<td>42.27 ± 0.82</td>
<td>16.45 ± 0.68</td>
<td>13.2 ± 0.12</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
<td>(c)</td>
<td>(c)</td>
<td>(a)</td>
<td>(a)</td>
</tr>
<tr>
<td>05-PI-6000-2.3⁴</td>
<td>6.08 ± 1.64</td>
<td>42.46 ± 1.19</td>
<td>16.29 ± 0.61</td>
<td>13.37 ± 1.01</td>
<td>0.44 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(c)</td>
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</tbody>
</table>

1. Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin
2. Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin
3. 05-PI-6000-1.2: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated with 1.2 kGy
4. 05-PI-6000-2.3: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin and irradiated with 2.3 kGy

Means in the same column with different letters a–c are significantly (P < 0.05) different.
**Effects of selected treatments on sensory quality.** Sensory panelists evaluated frankfurters for visual purge, visual color, texture and specific preselected aroma notes. The results are shown in Table 2. The purge assessment again reflects the volume of added solutions, such as the 2 ml of pediocin (Pdn-6000). The control was significantly (P < 0.001) lower for apparent purge than the treatments because there was no added solution. There was no significant (P > 0.05) difference in purge between irradiated frankfurters and those subjected to other treatments, once the effect of added solutions was removed.

Color scores for the frankfurters (Table 2) were lower for the irradiated treatment group compared with the control group. The irradiated frankfurters, however, were not different from the other treatments. Odor scores (Table 2), evaluated as smoky, burnt and acidic aroma, did not differ. Panel training included irradiated frankfurters so that odor notes and terminology could be established during training sessions with irradiated products. Panelists evaluated products that were exposed to a range of irradiation treatments to generate odor notes that might be detected in the experimental products. The experimental treatments did not result in significant (P > 0.05) changes in product odor.

The texture assessments of these samples by the sensory panel (Table 2) indicated that scores for the irradiation treatment were not different from the controls. The addition of pediocin increased the product resistance to slicing at the higher level of addition (Pdn-6000). This observation was surprising and a potential explanation is not clear. Instrumental evaluation of texture, however, showed no significant differences among the treatments or between the treatments and the control for either skin toughness or interior firmness of the frankfurters (Table 1).

As determined by subjective evaluations (Table 2), panelists could not (P > 0.05) distinguish any difference in texture, or in smoky, burnt and acidic aroma of samples after treatments. Purge was scored differently but this was due to the addition of solution as pointed out earlier. The results show that the treatments with pediocin and irradiation did not appreciably alter the texture scores (8.88-9.47), or smoky (6.41-6.92), burnt (2.22-2.30) and acidic (3.72-3.88) aroma scores compared to those of the control samples.

**Discussion**

**Effect of irradiation on L. monocytogenes**

Irradiation was effective for reducing *L. monocytogenes* on frankfurters and was more effective with increasing irradiation doses. The treatments irradiated at 2.3 kGy, regardless of pediocin concentrations added, provided better antilisterial effectiveness than at 1.2 kGy. Irradiation may have greater antilisterial effectiveness in cured meat products, such as cured hams (13) than uncured meats. The populations of *L. monocytogenes* (ca. 6 log CFU/g initial inoculation) on cured hams were reduced 2 logs or more by low-dose (0.90 kGy) irradiation, and to undetectable levels by medium-dose (2.0 kGy) irradiation (13). These authors suggested that a higher salt concentration (2.5%) and addition of nitrite to hams, vs. pumped pork chops with 1.0% salt, probably was responsible for the greater inhibition of *L. monocytogenes* on hams when low-dose irradiation was applied. The results from our study were consistent with those results. However,
Table 2. Sensory evaluation of frankfurters following selected treatments with pediocin (in ALTA™ 2341) and post-packaging irradiation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Purge</th>
<th>Color</th>
<th>Texture</th>
<th>Aroma Smoky</th>
<th>Aroma Burnt</th>
<th>Aroma Acidic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.29 ± 0.17</td>
<td>5.98 ± 0.55</td>
<td>7.10 ± 0.95</td>
<td>7.04 ± 0.50</td>
<td>1.60 ± 0.36</td>
<td>4.63 ± 0.04</td>
</tr>
<tr>
<td>(a)</td>
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<td>(a)</td>
<td>(a)</td>
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<td></td>
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<tr>
<td>05-Pdn-3000&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5.41 ± 1.73</td>
<td>6.99 ± 1.67</td>
<td>8.12 ± 1.03</td>
<td>7.46 ± 0.41</td>
<td>1.98 ± 0.37</td>
<td>4.75 ± 0.41</td>
</tr>
<tr>
<td>(b)</td>
<td>(a b)</td>
<td>(ab)</td>
<td></td>
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<tr>
<td>05-Pdn-6000&lt;sup&gt;3&lt;/sup&gt;</td>
<td>9.19 ± 0.39</td>
<td>8.42 ± 1.42</td>
<td>9.50 ± 1.14</td>
<td>7.41 ± 0.53</td>
<td>2.16 ± 0.30</td>
<td>4.35 ± 0.30</td>
</tr>
<tr>
<td>(c)</td>
<td>(ab)</td>
<td>(b)</td>
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</tr>
<tr>
<td>05-PI-6000-1.2&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9.74 ± 0.37</td>
<td>8.42 ± 0.68</td>
<td>8.88 ± 0.63</td>
<td>6.41 ± 0.08</td>
<td>2.22 ± 0.44</td>
<td>3.72 ± 0.79</td>
</tr>
<tr>
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<td>(b)</td>
<td>(ab)</td>
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<td></td>
<td></td>
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<tr>
<td>05-PI-6000-2.3&lt;sup&gt;5&lt;/sup&gt;</td>
<td>8.64 ± 0.76</td>
<td>9.05 ± 0.64</td>
<td>9.47 ± 0.35</td>
<td>6.92 ± 1.26</td>
<td>2.30 ± 0.57</td>
<td>3.88 ± 0.46</td>
</tr>
<tr>
<td>(c)</td>
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</table>

1. The numerical scales of sensory intensity used for purge, color, texture, aroma of smoky, burnt and acid were: 0 = none, extremely light, extremely soft, and none, respectively; and 15 = extremely abundant, dark, firm, and intense, respectively.
2. Pdn-3000: Frankfurters (5 links / pkg) treated with 3,000 AU pediocin.
3. Pdn-6000: Frankfurters (5 links / pkg) treated with 6,000 AU pediocin.
4. 05-PI-6000-1.2: Frankfurters (Slinks / pkg) treated with 6,000 AU pediocin and irradiated with 1.2 kGy.
5. 05-PI-6000-2.3: Frankfurters (Slinks / pkg) treated with 6,000 AU pediocin and irradiated with 2.3 kGy.

Means in the same column with different letters are significantly (P < 0.05) different.
recovery of *L. monocytogenes* in our study was more erratic which may have been due to the variation of actual absorbed doses during irradiation.

Because the formulation of frankfurters from different manufacturers may affect irradiation D-values for surface-inoculated *L. monocytogenes*, Sommers and Thayer (29) suggested that different brands of frankfurters may require doses from 2.45 to 3.55 kGy to achieve a 5-log reduction of *L. monocytogenes*. These results are also similar to the observations reported in our study.

**Synergistic effects between pediocin and irradiation**

The results of our study demonstrated the combined effects of pediocin and electron beam irradiation for effectively controlling or eliminating *L. monocytogenes* on frankfurters. There were synergistic interactions between pediocin (in ALTA™ 2341) and irradiation treatments. This may be explained by the following inferences. After addition of pediocin, the cell membrane of *L. monocytogenes* may be damaged, and may lose its integrity. Thereafter, the high-energy electrons from the electronic beam may more easily penetrate cells to directly damage DNA molecules or may further deconstruct the structure of the cell membranes by free radical attack. This action can effectively damage DNA molecules to achieve a greater impact than that of irradiation alone at the same doses without addition of pediocin.

**Effects of package types**

Although single-link packages had a slightly greater population reduction of *L. monocytogenes* than 5-link packages at both inoculation levels (3.40 and 5.20 log CFU/g), the package types did not significantly (P > 0.05) affect the treatment combinations of pediocin and post-packaging irradiation. From the evaluations during storage, little or no growth of this pathogen was observed in single-link and 5-link packages at 4°C. The samples showed erratic recovery when stored at 10°C or 25°C. The variation in range of absorbed doses may have resulted in variation of recovery of *L. monocytogenes*. Erratic recovery of damaged cells may also have contributed to the variation observed. The difference in antilisterial effects between single-link and 5-link packages was not significant (P > 0.05) during storage at 3 different temperatures.

**Effects of storage temperatures**

The lower refrigeration temperatures (4°C) were very important to control the growth of *L. monocytogenes* on frankfurters in this study. This is similar to previous observations (9, 10). Fu et al. (13) suggested that 7°C may be low enough to inhibit the growth of *L. monocytogenes* on either nonirradiated or irradiated (0.9 kGy) ham during 7 days of storage. It has also been reported that lower temperatures accompanied with pH (pH 6.0 or below), and sodium chloride can be important in controlling the growth of *L. monocytogenes* at lower populations (4 × 10² CFU/ml) (16). Therefore, the multiple hurdle concept incorporating refrigeration temperatures (4°C or 10°C) with the treatment combinations of pediocin (physicochemical pre-packaging barrier) and post-packaging irradiation (physical post-packaging barrier) provides a good strategy to control *L. monocytogenes* on RTE processed meat products. These treatment combinations were more effective than the combinations of pediocin with post-packaging thermal pasteurization (PPTP) (10) or the treatment with pediocin alone (9).
Fu et al. (13) simulated mishandling of products after purchase by storage of ham samples at 25°C for 2 days after storage at 7°C for 7 days. The results showed that temperature abuse accelerated the recovery of some injured cells of \textit{L. monocytogenes} on ham, even though this pathogen was reduced to virtually undetectable levels (initial inoculation of 6 log CFU/g) after irradiation with 2.0 kGy. Our study showed that the lower refrigeration temperatures, such as 4°C, enhanced the synergistic antilisterial effects of pediocin and irradiation.

\textbf{Antilisterial effect of multiple hurdle preservation}

We found that combinations of pediocin with post-packaging irradiation inhibited the growth of \textit{L. monocytogenes} throughout the entire storage period at refrigeration temperature (4°C). The single-link packages with added pediocin (Pdn-3000 or greater) combined with the irradiation at 2.3 kGy inhibited the growth of this pathogen at 4°C, 10°C or 25°C. Therefore, for frankfurters 3,000 AU of pediocin combined with a 2.3 kGy irradiation dose provides a good means to control \textit{L. monocytogenes}. We suggested previously (10) that the treatment combination of 6,000 AU of pediocin and PPTP treatment at 96°C/60s could be an economical and effective means to control \textit{L. monocytogenes} on RTE processed meat products. However, the treatment combinations of pediocin and irradiation were a more effective means to control \textit{L. monocytogenes} on frankfurters in this study.

Irradiation may provide an approach to reduce dependence on chemical additives because it decreases the microbial load and eliminates some food pathogens. It also decreases the opportunity for post-processing contamination if products are packaged before being irradiated (13). Fu et al. (13) demonstrated that irradiation was effective for reducing \textit{L. monocytogenes} on cured ham, especially at medium doses (1.8-2.0 kGy). Irradiation should ensure the safety of these products and should also extend their shelf-life under these multiple hurdle preservation treatments. Radomyski et al. (24) suggested that irradiation at doses up to 3.0 kGy are sufficient to eliminate most pathogens in meats. The data presented in this study demonstrated that irradiation as low as 2.3 kGy in combination with pediocin and storage at 4°C will inhibit the growth of \textit{L. monocytogenes} on frankfurters.

\textbf{Product quality analysis}

\textbf{Effects of irradiation doses.} Irradiation (0.75 to 2.0 kGy) did not affect pH, TBA, instrumental color or sensory color and odor attributes for chops or cured ham in previous studies (13). Generally, these results are similar to results of our study. Fu et al. (13) indicated that TBA value of most samples were within acceptable ranges (< 1.0), indicating product stability relative to lipid oxidation. The TBA values of irradiated samples in our study were also below 1.0. The authors also reported that irradiation as high as 1.8 kGy did not affect chemical changes, surface color, (Hunter L*, a*, and b* values and the sensory color score) and odor on ham. Several studies have suggested that irradiated frankfurters would be acceptable but others have reported impaired organoleptic properties (30, 31). These authors indicated that sensory traits (off-flavor, off-odor, texture and overall palatability) were less desirable in frankfurters irradiated at 32 kGy as compared with those irradiated at 8 kGy. Both levels of irradiation provided less desirable frankfurters than the controls (non-
irradiated or 0 kGy). However, the irradiation doses were very high. Another study reported that irradiation of
turkey frankfurters with doses of 5 to 10 kGy at two temperatures (2°C and -30°C) generally resulted in the
same product quality, with no difference in tenderness, freshness, off-flavor, or overall acceptability (3). The
irradiation doses used in that study did not appear to damage the sensory quality of the samples.

According to the intensity scores of sensory attributes in this study, the sensory quality of products was
not affected. The exception was purge accumulation and color, which were different (P < 0.05) for treated
versus control samples. The changes in these two sensory attributes were due primarily to the addition of the
solution containing pediocin.

Odor scores for irradiated samples were not significantly (P > 0.05) different from the control samples.
The physical and chemical analyses resulted in patterns similar to earlier studies (9, 10). The attributes of
samples treated with pediocin were not affected by combination with irradiation doses of 2.3 kGy or less. There
was no dose effect observed for TBA values and it appears that a dose of 2.3 kGy is not high enough to induce
lipid oxidation. This result also agreed with the results from the study of Fu et al. (13). Cured hams irradiated
at 1.8 kGy did not demonstrate lipid oxidation.

Effects of selected treatment combinations on physical and chemical attributes. As determined by
objective measurements, the results (Table 1) indicated that selected treatment combinations did not cause
appreciable untoward effects on the finished products. There was no significant (P > 0.05) difference on b*
value of Hunter L*-a*-b* values, firmness of skin and interior, and TBA values. However, purge accumulation,
L* and a* values of Hunter L*-a*-b* values, and pH values were significantly (P < 0.05) different compared to
the control group. Except for purge accumulation, the other measurements showed relatively small differences
compared to control group, even though statistical analyses indicated that the differences were significant (P <
0.05).

A large part of the apparent purge accumulation resulted from the original volume of 40% ALTATM
2341 suspension added to packages. The 5-link packages, for example, received 5 or 10 ml ALTATM 2341
suspension in total to achieve addition of 1 to 2 ml per link. The apparent purge accumulation from samples,
treated with Pdn-6000 and irradiation (5.38-6.08%), was significantly (P < 0.05) different from the control
samples (0.61%) and the samples treated with Pdn-3000 (3.33%). However, purge from the combined
treatment was not different (P > 0.05) from the samples treated with Pdn-6000 (5.13%) only (9). It is evident
that L* and a* color values, and pH values were affected by addition of ALTATM 2341 suspension. After the
treatments, the irradiated samples with 6,000 AU of pediocin were darker than control samples. Samples
injected with pediocin were also lower in pH though. The difference is small.

Conclusions

Treatment combinations of pediocin with post-packaging irradiation pasteurization of 2.3 kGy or
greater were observed to be effective antilisterial treatments. These combinations were very effective for
reducing the numbers of L. monocytogenes on frankfurters (single link, 5 links or 10 links per package) stored at
refrigerated temperatures (4°C or 10°C), and delayed growth of the remaining cells during storage. Storage at refrigeration temperature (4°C or 10°C) enhanced the antilisterial effects of these treatments. Thus, the storage temperature remains as a critical point to control this pathogen.

Irradiation not only had a large impact on *L. monocytogenes*, but also was synergistic with pediocin treatments to inhibit the growth of this pathogen to an even greater extent than when either was used alone. According to the intensity scores of sensory attributes, product quality was not affected. Thus, manufacturers could apply a combination of these hurdles as a CCP in their HACCP programs to improve control *L. monocytogenes* on frankfurters and the other RTE meat products.

**Acknowledgements**

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**References**


Conclusions

Antilisterial, and even listericidal, effects for treatment combinations of pediocin (Pdn-6000) and PPTP treatments (96°C for at least 60 s) or irradiation (2.3 kGy or greater) were observed. Results from this study showed that pediocin activity of the commercially available fermentation products in ALTA™ 2341 significantly reduced the numbers of L. monocytogenes on vacuum-packaged frankfurters. Treatments with pediocin alone or pediocin combined with thermal or irradiation pasteurization were effective for inhibiting the growth of L. monocytogenes on frankfurters stored at refrigeration temperatures, especially at 4°C, and delaying growth of the remaining cells during storage. Storage at 4°C enhanced the antilisterial effects of these treatments for improved safety and shelf-life of frankfurters. Irradiation and PPTP treatments both enhanced the antilisterial effects of pediocin but irradiation appeared to be synergistic with pediocin.

Selected treatment combinations of pediocin and irradiation or pediocin and PPTP treatments did not greatly affect physical and chemical properties or sensory attributes of the frankfurters treated. According to panel intensity scores for sensory attributes, product quality was not affected. Thus, manufacturers may utilize the combinations of these hurdles as a CCP in their HACCP programs to control L. monocytogenes on frankfurters and the other RTE processed meat products.

There is a specific implication from our results that smaller package types (< 5-link packages) allow greater antilisterial effects for PPTP treatments. Single-link packages generally resulted in the greatest reduction of L. monocytogenes counts following PPTP treatments.

Recommendations for Future Research

1. **Food safety application:** Use of pediocin to control L. monocytogenes by direct addition in the formula of RTE processed meat products should be investigated. Effect of pediocin on the other pathogens, such as E. coli O157: H7, Salmonella, etc. needs to be included at the same time.

2. **Food safety application:** Effects of pediocin and other barriers (PPTP or irradiation) to control L. monocytogenes after inoculation of L. monocytogenes at very lower level such as 20 CFU/g should be studied.

3. **Food safety application:** Use of treated casings, biodegradable films and co-extrusion technology offers easy means to deliver bacteriocins and other compounds to the surface of frankfurters. However, improvement of antimicrobial effectiveness is needed to make these systems useful.

4. **Food safety application:** Use of lactic acid bacteria inoculated on samples or in packages to provide a safety indicator for meat products may have potential to prevent pathogen growth.
5. **Food safety application:** Effects of antioxidants, such as erythorbate, vitamin E, rosemary or BHA/BHT on inhibitory effects of irradiation against *L. monocytogenes* should be studied to determine the effects of antioxidants in protecting bacteria or pathogens from antimicrobials or other inhibitory treatments.

6. **Food safety application:** Effects of pediocin, PPTP, irradiation, and other treatments on adaptation of *L. monocytogenes* to sublethal stresses protect that this pathogen from subsequent inactivation treatments.

7. **Food safety application:** Comparison of inhibitory effects on *L. monocytogenes* of single-pass and double-pass irradiation treatments at the similar absorbed doses should be investigated.
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