PRE- AND POST-PACKAGE PASTEURIZATION OF READY-TO-EAT MEATS TO CONTROL *LISTERIA MONOCYTOGENES*

By

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Bachelor of Technology

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2001

Submitted to the faculty of the
Graduate college of
Oklahoma State University
in partial fulfillment of the
requirements for
the degree of
MASTER OF SCIENCE
December, 2003

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READY-TO-EAT MEATS TO CONTROL

LISTERIA MONOCYTOGENES

Thesis Approved:

Thesis Advisor

Dean of the Graduate College

PREFACE

This research was conducted to provide support for the effectiveness of new approaches in microbial intervention for the control *Listeria monocytogenes* on ready-to-eat meats (roast beef, turkey bologna and ham). Specific objectives of this research were to a) apply radiant heat oven for pre-package surface pasteurization to reduce *Listeria monocytogenes*, and b) apply pre-package pasteurization in combination with post-package pasteurization (using submerged hot water bath) to control *Listeria monocytogenes* on ready-to-eat meats.

I sincerely thank my major advisor Dr. Peter M. Muriana for the guidance and support in the completion of this project.

ACKNOWLEDGEMENTS

I sincerely express my appreciation to my major advisor Dr. Peter M. Muriana for his guidance, supervision and understanding nature. My appreciation also extends to other committee members Dr. William McGlynn and Dr. Christina Dewitt for the guidance and encouragement all along the course of my study. I will like to thank Dr. Peter M. Muriana, the Department of Animal Science and Food and Agricultural Products Research Center (FAPC) for providing me with this research opportunity and financial support.

I would like to thank the student members of the pathogenic microbiology lab, and our sponsor companies for providing technical and product support in conducting this research. I thank my parents for their encouragement and support.

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NOMENCLATURE

Hr	hours
Mg	milligrams
Ml	milliliter
In	inch
µm	micrometer
mm	millimeter
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
TSA	Tryptic Soy Agar
LPS	Lipopolysaccharide
MPA	monocyte-producing agent
LRR	Leucine-rich repeat
Ca^{+2}	Calcium
LLO	Listeriolysin-O
RTE	ready-to-eat
FSIS	Food Safety and Inspection Service
USDA	United States Department of Agriculture
CDC	Center for disease control
Aw	water activity

QAC	Quaternary Ammonium Compounds
HPP	High Pressure Processing
Mpa	MegaPascal
UV	Ultraviolet
KGy	KiloGrey
GRAS	Generally Recognized as Safe
EPA	Environmental Protection Agency
CFR	Code of Federal Regulations
CFU	Colony forming units
MDA	Malonaldehyde
PUFA	Polyunsaturated fatty acids

CHAPTER I

INTRODUCTION

1.1. Background- *Listeria and L. monocytogenes*

A small gram-positive bacillus causing monocytosis was isolated from the infected blood of a rabbit and the causative organism was named *Bacterium monocytogenes* (Murray et al., 1926). Murray et al. (1926) are generally credited for their description of the causative agent of listeriosis. But reports suggesting the incidence of listeriosis like symptoms can be dated to as early as 1891. Sometime after the isolation of *Bacterium monocytogenes* by Murray et al. (1926), Pirie et al. (1940) isolated a bacterium from a gerbille (an African jumping mouse) in South Africa and named it after Lord Lister as *Listerella hepatolytica*. The name was changed to *Listerella monocytogenes* after it was discovered that the characteristics of *Bacterium monocytogenes* and *Listerella hepatolytica* were the same. In 1931 at the 3rd International congress for microbiologists, it was reported that the name *Listerella*, refers to a group of slimy molds given by Jahn in 1906, so the name was changed to *Listeria monocytogenes* (Pirie et al., 1940).

1.2. Taxonomical classification:

In 1985, eight different species were recognized under the genus *Listeria*. These include *L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, L. *denitrificans*, *L. murrayi* and *L. grayi* (Jones and Seeliger, 1986). Based on DNA-DNA hybridization studies it was found that *L. murrayi* and *L. grayi* are not distinctly different from each other and so was included under a single species *L. grayi*. Also, the species *L. denitrificans* was found to be closely

related to the genera *Cornybacterium, Cellulomonas* and therefore was moved to another new genus *Jonesia* as *J. denitrificans*. Due to the above controversial reports, these species are listed as species of uncertain affiliation/position in the Bergey's Manual of Systematic Bacteriology (Ryser et al., 1991a).

1.3. General characteristics : *Listeria spp.* And *Listeria monocytogenes*:

The 6 species belonging to the genus *Listeria* are short rods that are 0.4-0.5μm in diameter and 0.5-2 μm length with rounded ends and can occur either singly or as short chains. They are gram-positive, non-spore forming, facultatively anaerobic and not encapsulated. Motility is seen when grown in the temperature range of 20-25°C while the optimum temperature for growth is 30-37°C. They also exhibit fermentative metabolism of glucose. They can survive temperatures below freezing to body temperatures and the pH range optimum for growth is 5-9.6 (Ryser et al., 1991a).

Of the 6 species, *L. monocytogenes* is the only one that causes severe human diseases. (Novak et al., 2003). The characteristics which make it a formidable pathogen are its ability to grow at low temperatures (psychrotroph), ubiquitous nature, ability to form biofilms on food processing equipments, its heat tolerance is greater than other foodborne pathogens, it can survive conditions like freezing and drying, is salt resistant (nitrite), causes listeriosis and has a high mortality rate (25-30%). Thus its ability to survive and grow in such diverse conditions is one of the many challenges posed by this organism. Excellent growth occurs after partial replacement of oxygen with carbon dioxide but it fails

to grow under strict anaerobic conditions. Therefore it is considered to be an aerobically growing and facultatively anaerobic organism (Ryser et al., 1991a).

1.4. Cultural characteristics:

L. monocytogenes can grow in a temperature range of 1-45°C, but the optimum growth temperature is 30-37°C. The organism can grow on common bacteriological media like Tryptose Agar and can also be stored in the same media at 3°C. Typical colonies of *L. monocytogenes* observed after 24 hrs of incubation time are 0.3-1.5 mm in diameter, round, translucent and have slightly raised colonies with a finely textured surface. *L. monocytogenes* can also grow on blood agar and form a narrow zone of β-hemolysis around the colonies after 48 hrs of incubation at 37°C (Ryser et al., 1991a).

1.5. Biochemical profile:

All *Listeria* spp. Are catalase positive, oxidase negative, urease negative, methyl red/voges-proskauer test positive. They ferment glucose and produce acid but not gas from glucose, esculin or maltose. An important characteristic of these species is their inability to hydrolyze gelatin, casein or milk. Rhamnose and xylose reactions are specific in differentiating different *Listeria* isolates. *L. monocytogenes* produces acid from rhamnose and so do some strains of *L. innocua, L. welshimeri.* Species *L. ivanovii, L. welshimeri, L. seeligeri* use xylose and produce acid without gas. Mannitol is only used by the species *L. grayi* (Doyle, 1989).

Different species of *Listeria* can be differentiated according to the biochemical tests listed in Table 1. All the species produce hemolysis on blood

agar. The CAMP test (which was first discovered by Christie, Atkins and Munch-Peterson in *Streptococci*) is of particular importance in differentiating species of *Listeria*, i.e. *L. monocytogenes*, *L. seeligeri*, *L. ivanovii* (Ryser et al., 1991a). The method (Fig.1) involves streaking of *Staphylococcus aureus* and *Rhodococcus equi* vertically on a plate of sheep blood agar. Cultures of *Listeria* are then streaked at right angles to the above-mentioned cultures and incubated for 48hours at 35°C. The zone of β-hemolysis by *L. monocytogenes* is enhanced in the presence of *S. aureus* while that of *L. ivanovii* is enhanced by *R. equi*. Enhanced hemolytic activity is also shown by *L. seeligerii* in the presence of *S. aureus*, but is weakly hemolytic when compared to *L. monocytogenes*. Other species like *L .innocua* remain non-hemolytic (Ryser et al., 1991a).

Table 1. Biochemical characteristics of different species of *Listeria* (From Ryser et al., 1991a).

	Listeria sp.							
Biochemial test	*Monocytogenes*	*Ivanovi*	*Innocua*	*Welshimeri*	*Seeligeri*	*Grayi*	*Murrayi*	*denitrificans*[a]
dextrose	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	+	+
Rhamnose	+	-	V[b]	V	-	-	V	-
Xylose	-	+	-	+	+	-	V	-
Hippurate hydrolysis	+	+	+	+	+	-	-	-
Voges-proskauer	+	+	+	+	+	+	+	-
Methyl red	+	+	+	+	+	+	+	+
Beta-hemolysis	+	+	-	-	V	-	-	-
Urea hydrolysis	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	+	+
Catalase	+	+	+	+	+	+	+	+
H_2S on TSI	-	-	-	-	-	-	-	-
H_2S by lead acetate strip	-	-	-	-	-	+	+	-
CAMPpositive*S.aureus*	+	-	-	-	+	-	-	-
CAMP positive/R. equi	-	+	-	-	-	-	-	-

[a]Reclassified as *Jonesia denitrificans*
[b]V- Variable

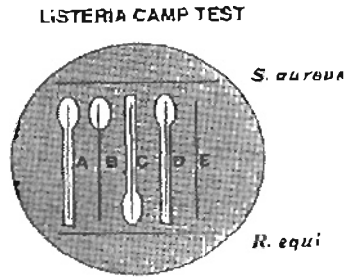

Figure1. CAMP test to differentiate species of *Listeria*

The white zone of β-hemolysis within the dark circle indicates positive reaction. A-*L. monocytogenes* (typical), B- *L. monocytogenes* (atypical), C- *L. ivanovii*, D- *L. seeligeri*, E- *L. innocua* (Ryser et al., 1991a).

1.6. Serology:

Serological classification is a valuable tool for identifying different isolates of *Listeria*. Paterson (1940) described the serological types of *Listeria* based on both somatic (O) (from the German *ohne Hauch*) and flagellar (H) (from the German *Hauch*) antigens and divided them into 4serotypes. Serotypes 1, 3, 4 were identified on the basis of O-antigens while serotype 2 was based on a unique H-antigen. Seeliger (1961) later splitted serotype 4 into 4a and 4b. *L. monocytogenes* isolated from pathological sources are most likely to be 1/2a, 1/2b, 1/2c, 3a, 3b, 3c and 4d. (Doyle, 1989). Studies by Ryser et al. (1991a) showed that O and H antigens of *Listeria* are complex carbohydrate-containing

proteins with partially overlapping and specific fractions. It was found that O-antigens are heat stable while H-antigens are heat labile.

The classification of *Listeria* spp. can also be based on their O and H antigenic structures. *L. innocua, L. ivanovii, L. seeligeri* exhibit one or more somatic antigenic structures similar to *L. monocytogenes*, whereas *L. grayi* and *L. murrayi* can be differentiated from the rest of *Listeria* species based on their H-antigen. Thorough biochemical characterization in combination with serotyping is required to sufficiently differentiate *L. monocyotgenes* from other *Listeria* species.

1.7. Nutritional requirements:

L. monocytogenes is a non-fastidious organism and can grow in simple media. The ideal carbohydrate for growth is glucose and it cannot be replaced by other carbon sources like xylose, arabinose or ribose as a source of energy. Substances obtained from citric acid and pyruvate cycles are also not suitable for its growth (Ryser et al., 1991a). Media containing low amount of growth factors can support growth of *L. monocytogenes* when enriched with blood and serum although they are not required for growth. Different strains have different requirements for vitamin B while some require both pyridoxine and riboflavin. (Gray, 1966). Media containing glucose, riboflavin and biotin together with the amino acids isoleucine, leucine, valine and cysteine support growth of *L. monocytogenes*. (Ryser et al., 1991a). Iron, magnesium, calcium and potassium are required for the growth of *L. monocytogenes*. Increased levels of thiamine are required to obtain colonies of reasonable size. Riboflavin is required when iron is added as a supplement to enhance growth of *L. monocytogenes*.

1.8. Modes of Transmission:

Due to the ubiquitous nature of the organism, humans can come into contact with the pathogen through a variety of sources such as animals, raw milk, dairy products, sea foods, processing plants, air, water and dirt as shown in Fig.2 (Ryser et al., 1991b).

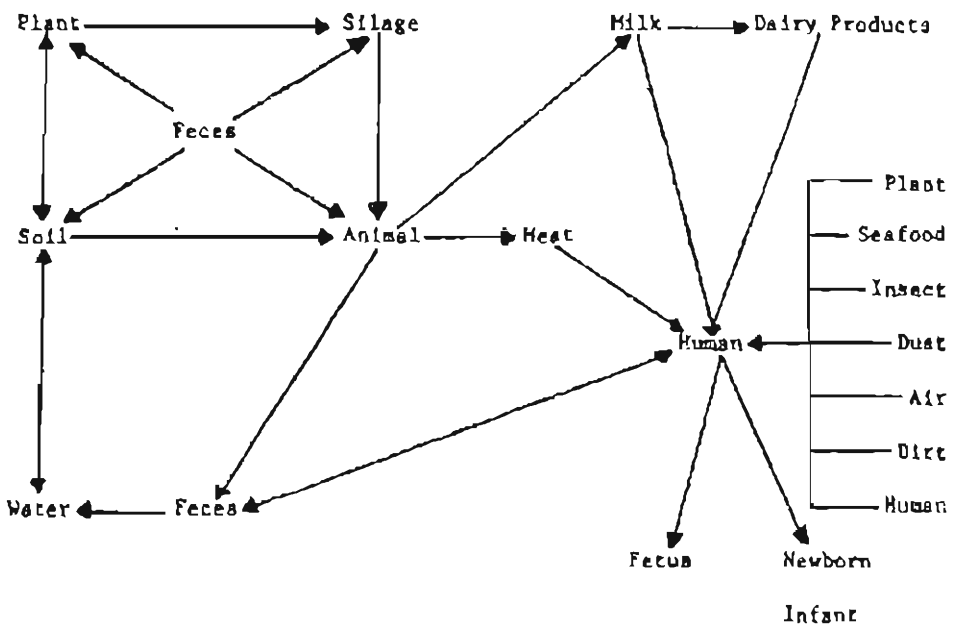

Figure 2. Transmission of *Listeria monocytogenes* to humans (Ryser et al., 1991b).

Among dairy products raw milk, pasteurized milk, cheese and butter has been examined intensively because of its known association with foodborne listeriosis. (Farber et al., 1991). Because of the widespread and psychrotropic nature of the organism in meat processing and packaging environments, incidence of *L. monocytogenes* is of primary concern. The organism can be acquired by product during cutting, slicing, packaging or from cross-

contamination from the carcass. The incidence of *L. monocytogenes* in meat varies and may be as high as 92% (Vignolo et al., 1996). It is also present in processed and refrigerated RTE meat products like sausages and vacuum packaged meats. Presence of this pathogen in products that are usually consumed without reheating is of particular concern. Contamination of the RTE or fully cooked meat can occur when opened and handled further. The same method of contamination was observed in poultry processing environments. Cross-contamination between raw and finished product is one of the major sources by which seafoods and fish get contaminated. Epidemiological studies indicate that a major source of contamination might be cross-contamination that occurs in processing plants (Thompkin, 2002). Niches in the working areas such as walls, floors, ceilings, condensate, processing waste, drains, wash areas, and other food contact surfaces may harbor the pathogen which can contaminate food products. Mechanical equipment contaminated with *L. monocytogenes* in abattoirs has also been identified as the source of contamination in a pig slaughterhouse in Finland (Autio et al., 2000). Studies on the prevalence of *L. monocytogenes* showed that 16% of raw pork and 17% of raw poultry meats are contaminated (Chasseingnaux et al., 2002).

A surveillance program conducted by FDA indicated the association of *L. monocytogenes* with domestic and imported cheese, ice cream and other dairy products has also lead to numerous recalls. This can be due to the presence *L. monocytogenes* in raw milk. However effective pasteurization treatment can control this pathogen. (Kazak et al., 1996). Vegetables like cabbage, celery,

tomatoes and lettuce can also serve as a vehicle for listeriosis as indicated by an outbreak linked to the consumption of coleslaw in 1981 in Canada (Ryser et al., 1991b). Plant parts and vegetables used as salad vegetables play an important role in transferring the pathogen from the natural habitat to the human food supply.

Formation of biofilms can also be a source of contamination. *L. monocytogenes* has been found to form biofilms on food contact surfaces like plastics, polypropylene, rubber, stainless steel and glass (Herald et al., 1988). About 40% of foodborne outbreaks occurred in France in 1996 were linked to contamination of processing equipment, thereby due to biofilm formation by the pathogen (Midelet et al., 2002). Microorganisms developing biofilm can form a complex multicellular structure and surround themselves with an increased exopolysaccharide, which protects the organism against sanitizers. Thus they can escape and can attach to the surface of foods (Chae et al., 2001). Surface water can also be a source of contamination (Doyle et al., 1989). Though complete elimination of *L. monocytogenes* is nearly impossible, the likelihood of producing *L. monocytogenes* contaminated food products can be greatly reduced by following Good manufacturing practices (GMP). Regular cleaning and sanitizing of equipments during manufacturing and packaging of food products is required.

CHAPTER II

LITERATURE REVIEW – *L. MONOCYTOGENES*

2.1. Morphological Characteristics:

The important morphological characteristics are

Motility. *L. monocytogenes* is motile via flagella. It may be either monotrichous (single polar flagellum) or may pocess peritrichous flagella (flagella distributed over the entire cell). Motility is seen only at lower temperature (20°C) and at higher temperatures around 37°C a reversible damage of the flagella is seen. This leads either to the development of a single flagellum or immobility. Electronmicroscopic studies of the flagella by Leifson et al. (1955) showed peritrichous, spiral, coiled and straight forms. (Fig-3). The motion starts with rotations and wiggling movements to fast excentric rotations before the organism heads in a definite direction. *Listeria* also exhibits a characteristic tumbling movement, which aids in identification and can be seen in Tryptose Broth cultures incubated at 20°C.

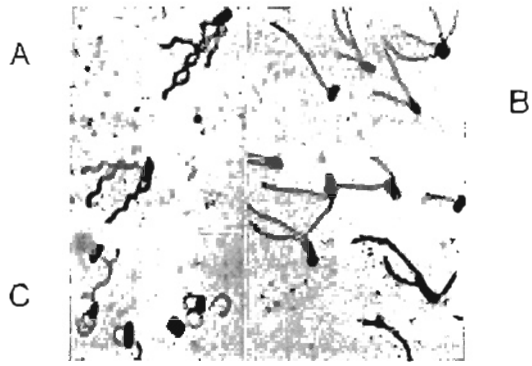

Figure 3. Flagellation of *L. monocytogenes*

A- peritrichous flagella in spirals B- partly peritrichous, partly monotrichous straight flagella, C- short coiled flagella (Leifson, et al., 1955)

Toxin Production. Liu et al. (1961) first published the production of toxin by *L. monocytogenes* in 1961. They succeeded in demonstrating toxin in sterile filtrates obtained from cultures grown in Trypticase Soy broth. This toxin was capable of producing hemorrhagic lesions within 3 hours after injecting intracutaneously into test rabbits. The lesions observed after 18 hours were necrotic and were similar in nature those produced by living cultures. This toxin can be inactivated when heated at 70°C for 30 min and was not precipitated with 30% saturated ammonium sulfate.

In general pathogenic *Listeria* spp. produce variety of endotoxins that can cause membrane damage and lysis. A toxic polysaccharide from *L. monocytogenes* grown on Typticase Soy agar was obtained by treating the cells with phenol and further fractionation with absolute alcohol, sodium acetate and acetic acid causing skin reactions in rabbits (Robinson et al., 1964). McIlwain et

al. (1964) isolated 3 proteinaceous fractions from disrupted *L. monocytogenes* cells that increased the respiration rate, body temperature, hyperglycemia followed by hypoglycemia and lead to the depletion of blood sugar level leading to death when injected into test animals. A significant increase in monocytes was observed within 48hrs of injection of these extracts. It was also observed that rabbits injected with toxic cellular protein fractions developed monocytosis much earlier than animals injected with viable *L. monocytogenes.* Ripo et al. (1995) identified the involvement of a sulphydryl-activated toxin in hemolysis and concluded that proteins responsible for hemolysis play an important role as virulent determinants.

Monocyte Producing Properties. All *L. monocyotgenes* spp. possess the ability to produce monocytosis and can be seen irrespective of their serological and biochemical differences. It is this property of *Listeria monocytogenes* that was used in naming the species (Ryser et al., 1991a). Stanley (1949) discovered the monocyte-producing agent (MPA) in non-antigenic, non-toxic, chloroform-soluble lipid extracted from the bacterial cell. It was suggested that MPA might be similar to that of an exotoxin that is stimulated by the presence of the bacterial cell in the animal body. He finally concluded that although monocytes do not produce any antibodies, they play an important role in the transport of antibodies, which help in the defense mechanism of the animal against the organism.

2.2. Pathogenesis. *L. monocytogenes* is a highly complex organism that has evolved a variety of tools and mechanisms to establish a successful infection after ingestion of the contaminated food. Infection starts with internalization of

the bacterium via phagocytosis in the case of macrophages or induced phagocytosis in nonphagocytic cells such as epithelial, endothelial, or hepatocytic cells (Cossart et al., 2001). The steps employed by the bacterium during its intracellular life cycle (Fig-4) are

Figure 4. Steps employed by the bacterium during intracellular life cycle (Vazquez-Boland et al., 2001).

1) entry into the host cell, 2) formation of phagocytic vacuole, 3) lysis and escape from the phagocytic vacuole, 4) liberation of the bacterium into the cytoplasm followed by multiplication, 5) actin-polymerisation that provides the propulsion for intracellular movement, 6) formation of pseudopods (listeriopods) that invaginate into the neighboring cell wall and thus initiate cell-to-cell spread, 7) survival in the secondary phagosome 8) escape from the secondary phagosome, and reinitiating of the cycle (Vazquez-Boland et al., 2001). Thus the bacterium disseminates in the host tissues by cell-cell spread and is simultaneously protected from host antibodies. The bacterium then disseminates from the epithelial cells (intestine) to the blood, inner organs, liver and eventually to the brain and foetoplacental barriers. Therefore, in order to produce infection,

the organism must survive conditions like acidity of the stomach, penetrate the intestinal lining and finally grow in the environment of the host cells. In order to accomplish these tasks, the organism exhibits several virulence genes. Many virulence factors responsible for the intracellular parasitic life-cycle have been identified. Vazquez-Boland et al. (2001) characterized these virulence factors to be associated with genes that are physically linked in a 9-kb chromosomal "virulence island". Cossart (2002) recently identified '*bsh*' gene, a virulent gene in *L. monocytogenes* that confers resistance against bile salts in the intestine. Some of the important factors that contribute to the pathogenicity of the organism include formation of Listeriolysin O (LLO), expressions of various virulence genes, and stimulation of monocyte production (Cabanes et al., 2002).

2.2.1. Bacterium-induced phagocytosis:

Entry of the bacterium into mammalian cells is mediated by a wide variety of components of the cell wall. The cell wall serves as a site of proteins that are beneficial for the organism and play an important role in bacterial adherence, invasion and interaction with the host immune system (Cabanes et al., 2002). The bacterium is taken up directly by professional phagocytic cells (i.e. macrophages) or by induced phagocytosis by the activity of membrane proteins in the case of nonphagocytic cells.

2.2.2. Bacteria- host interactions involved in entry:

Two listerial surface proteins, Internalin A (InlA) and Internalin B (InlB), mediate entry of the bacterium into the host cells (Fig.5). Both of these proteins belong to a large group of surface-exposed leucine-rich repeat (LRR) proteins

identified in the genome at the amino (N)-terminal but differ in their carboxyl (C)-termini (Schubert et al., 2002). Internalin A is a signal peptide, has a C-terminal region containing LPXTG peptide (X- can be any amino acid). InlA functions as an invasion protein, mediating internalization of the bacterium by the human epithelial cells. Its target/cell receptor in the epithelial cells is E-Cadherin. Cadherins are glycoproteins and have 5 extracellular domains, a membrane-spanning region, and a highly conserved cytoplasmic domain, which acts like a bridge between cadherin and the actin cytoskeleton (necessary for motility) via catenins (Brown et al., 2000). Entry of *L. monocytogenes* into host cells mediated by internalin requires a proline residue at position 16 of E-Cadherin and is responsible for the host specificity of the virulent forms of *Listeria*. This binding of InlA-E-Cadherin is important for the entry of the bacterium (Carbanes et al., 2002). It is assumed that the concentration of Ca^{+2} in the intestinal compartment is around 2mM, which is sufficient to allow recognition and binding of InlA to E-Cadherin followed by phagocytosis.

InlB is also a surface protein similar to InlA with a signal peptide and LRR but does not possess an LPXTG motif. Brown et al., (2000) stated that InlB binds Ca^{+2} in an unusually exposed manner and suggest that Ca^{+2} may act as a bridge between InlB and host cell surface receptors. Four other internalin like proteins, InlE, InlF, InlG and InlH were identified by Carbanes et al. (2002) and found not to be involved in invasion but were considered important for colonization of the host tissue. Thus the abundance of surface proteins and the variety of anchoring systems may be related to the ability of the organism to

survive under various environmental conditions and also interact with a large variety of cell types.

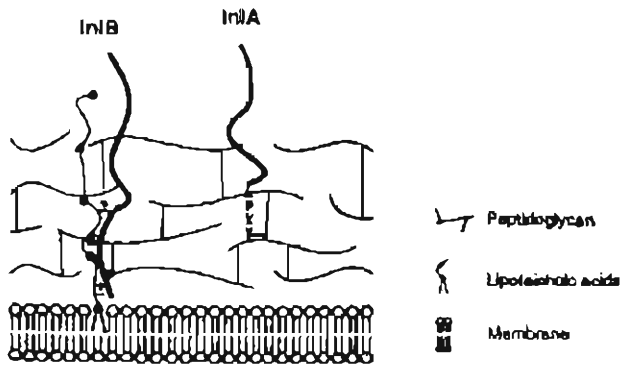

Figure 5. Surface proteins InlA and InlB (Braun et al., 2000).

2.2.3. Lysis of the phagocytic vacuole:

After internalization of the bacterium via phagocytosis, the bacterium mediates lysis of the vacuole by a pore-forming bacterial toxin, Listeriolysin-O (LLO), after about 30 min. (Fig.6). LLO is optimally active at pH 5.5-6 and is less active at higher pH. The bacterium then multiplies in the cytoplasm with a doubling time of approximately 1hour (Cossart et al., 2003). Puncture of the vacuolar membrane also reduces the concentration of Ca^{+2} to cytosolic levels and this reduces the affinity of InlA for E-Cadherin, thus helping the release of the bacterium from the vacuole to the cytoplasm. LLO also plays a role in the cell-to-cell spread of the bacterium (Braun et al., 2000).

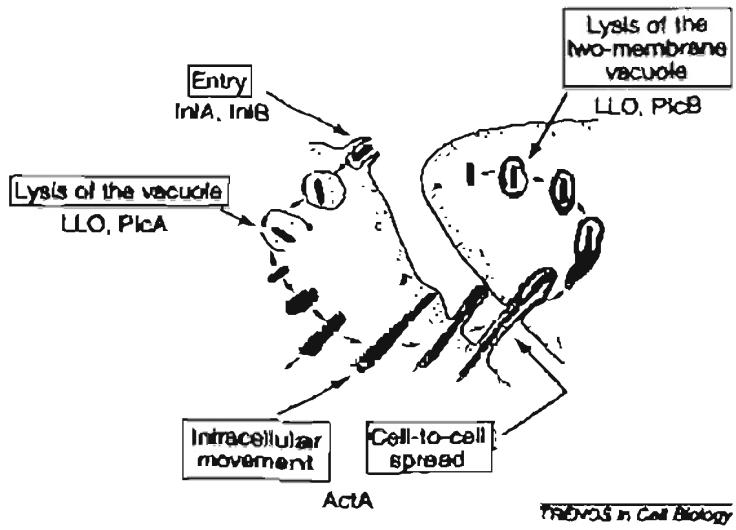

Figure 6. Factors involved during various stages of entry of the pathogen into the host cell (Cossart et al., 2003).

2.2.4. Actin-based motility and cell-cell spread:

Once the bacterium enters the cytoplasm, apart from multiplying the bacterium also gets associated with actin filaments. It may take about 2 hours for the filaments to get rearranged at one pole of the bacterium into "tails", which propel the bacterium in the cytosol by extension of the actin filaments. When *Listeria* reaches the plasma membrane, it induces the formation of a protrusion containing the bacterium. This protrusion invaginates into the neighboring cell and induces a second membrane around the cell. This membrane is further lysed and the bacterium is released thereby infecting the cytoplasm of the second cell (Fig.6) (Braun et al., 2000). ActA is a cell-surface protein that helps to induce actin-based motility. It can be divided into 3 regions, a highly charged amino terminus (ActA-N), a central domain consisting of proline-rich repeats

(PRR) and a carboxyl terminus, which upon deletion doesn't affect the motility of the organism (Cossart et al., 2001). ActA through its central domain recruit VASP (vasodilator-stimulated phosphoprotein) protein that is found in sites of active actin-polymerization. ActA-N is essential for the movement of bacterium and together with a complex of polypepetides, along with Arp2 and Arp3 can nucleate actin and generate a network of actin filaments. (Fig.7).

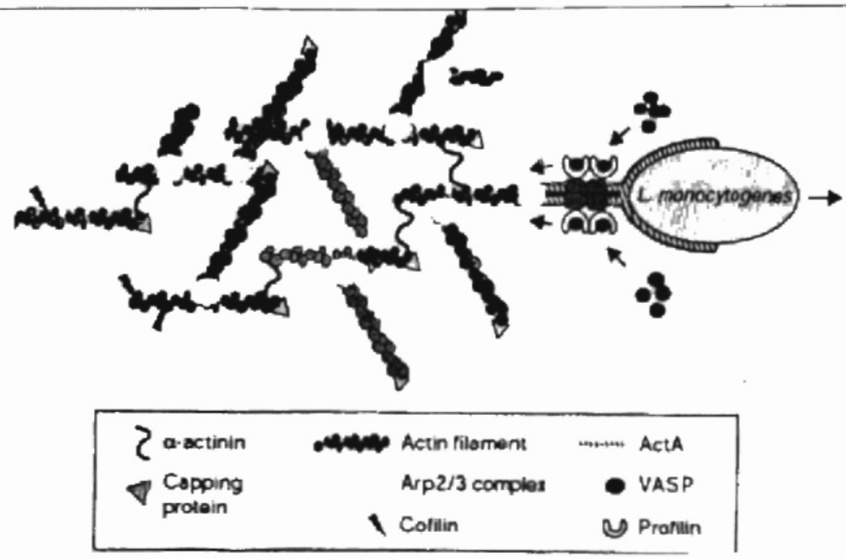

Figure 7. Actin assembly induced by ActA (Cossart et al., 2001).

2.2.5. Virulence gene cluster:

The virulence locus consists of 3 transcriptional units (Fig. 8). The central position being occupied by *hly* monocistron encodes for the synthesis of the pore-forming hemolysin, listeriolysin (LLO). The *L. monocytogenes* hemolysin

has been designated as either LLO oy Hly in the literature. LLO is a cholesterol binding, thiol-activated hemolysin and is required for disruption of the phagocytic vacuole and release of the bacterium into thecytosol. Absence of the virulence factor Hly leads to avirulent nature of the organism (Ermolaeva, 2001). Downstream to *hly* is a 5.7kb operon comprising *mpl, act*A and *pcl*B. The *act*A gene encodes for ActA protein which is required for motility and cell-cell spread of the bacterium. The gene *pcl*B, encodes a zinc-dependent phospholipase C (PlcB), which cooperates with Hly in the lysis of the phagosome and the export of the bacterium into the cytoplasm. But the principal function of PlcB is to mediate dissolution of the double-membrane secondary phagosmes formed after cell-cell spread. *Inl*A and *Inl*B genes encode for InlA and InlB that are essential for the induction of phagocytosis. Upstream and divergent from *hly* lies *plc*A-*prf*A operon. The first gene encodes for the synthesis of PlcA, a phosphatidyllinositol-specific phospholipase C that also helps in the lysis of phagosome and escape of the bacterium. The second gene encodes for PrfA protein. PrfA is required for the transcriptional activation of all the genes of the cluster including *prf*A. It is the only virulence regulator identified in *Listeria*.

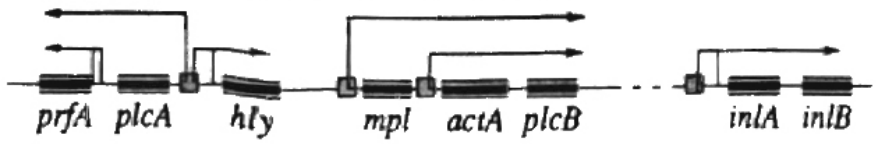

Figure 8. Gene Cluster of *L. monocytogenes*

Squares denote PrfA binding sites, transcriptions dependent on PrfA are denoted by arrows in the squares and those independent are denoted by arrows not connected to the squares. (Ermolaeva, 2001).

A number of environmental and growth phase dependent signals control the expression of virulence genes via PrfA. The *plc*a gene and genes of the *mpl-acta* operon are entirely controlled by PrfA regulatory protein. Hly and InlAB are only partially *prf*A-dependent and can be transcribed from the *prf*A-independent promoters (Ermolaeva, 2001; Hanawa et al., 2002).

2.3. Resistance *to L. monocytogenes* infection:

After the consumption of food contaminated with *L. monocytogenes*, the organism may pass through the walls of the digestive tract, and then possibly the liver, circulatory system and finally the central nervous system. Listeriosis is of primary concern to the population including pregnant women, newborns and immuno-compromised adults. Ryser et al. (1991c) analyzed the early stages of infection by injecting mice with a sublethal dose of *L. monocytogenes*. This study showed that within a period of 10 min after injection the organisms were captured by the liver and the spleen and subjected to antibacterial activity. Macrophages also known as kupper cells killed 90% of the injected organisms while cells that

escaped from macrophages grew exponentially and reached a maximum population within 2-3 days. The resistance against infection can be either due to T-cells, T-cell independent mechanisms, or by acquired immunity (Harty et al., 1996). Unanue (1997) identified involvement of cytokines such as interleukin (IL)-12 and tumor necrosis factor (TNF)-α, which are produced by *L. monocytogenes*-infected macrophages. These cytokines in turn stimulate production of interferon (INF) γ and natural killer (NK) cells. These are further responsible for cytocidal activity and curbing the growth of organism (Goldfine et al., 2002).

T-lymphocytes that lead to acquired immunity are activated when the cells are infected with the organism. A 2-stage response involving the priming of prekiller T-cells and the generation of *listeria* antigen-specific helper T-cells is then activated. As a result, a soluble substance (IL-2) converts pre-killer T-cells into *L. monocytogenes*-dependent cytotoxin and a T-lymphocyte is generated. This rapidly destroys the organism (Ryser et al., 1991c). Cytotoxin TNF-α plays an important role in the activation of macrophages and also increases the expression of adhesion molecules required for the action of neutrophils. Neutrophils curb the spread of the organism in the liver and thus control the spread of the infection (Unanue, 1997). The neutrophils also surround the hepatocytes, which are infected during the early stages of infection and act on the organism when released due to the apoptosis program of hepatocytes. Thus a synergistic interaction of T-cell independent and dependent processes is required for resistance against listeria infection.

2.4. Listeriosis:

Listeriosis is a sporadic disease caused by *L. monocytogenes* and is seen throughout the year although the incidence is higher during warmer climates and has a worldwide prevalence. Listeriosis is clinically defined when the organism is obtained from blood, cerebrospinal fluid or the placenta. Isolation of the organism from pregnant women or from her offspring within the first 31 days of delivery constitutes a perinatal case (Tappero et al., 1995). Wide ranges of food products like milk, soft cheese, ready-to-eat meats (bologna, turkey, sausage, luncheon meats, roast beef, ham salad, chicken salad, chicken turkey franks) and vegetables are implicated with listeriosis (Morritt et al., 2002). Clinical manifestations of Listeriosis were first published during the 1920's (Schlech, 2000). The global distribution of listeriosis among animals is analogous to the cases of listeriosis in humans all over the world. There is no particular sex preference with the exception that pregnant women are more susceptible. Also there is no strict relation of the occurrence with age except some clinical manifestations that show certain differences related to age. Due to the relative immaturity of the newborn and juvenile body, they are subjected to many bacterial infections like listeriosis. In contrast an adult body has protection against infection for the first few days because of the specific immunity stimulated by the defense mechanisms. But the case fatality is high among adults.

Although food is considered to be the primary source of infection, it is not the only mode of transmission. A study by McLauchlin (1996) showed that

Listeriosis can be transmitted as cutaneous lesions when the farmer or a veterinarian comes in contact with the infected animal. Transmission can also occur as cross-infection between newborn infants. This was seen when the infants born with congenital Listeriosis are delivered or nursed in the same or adjacent room along with other infants.

2.4.1. Characteristics and risk groups of Listeriosis:

In the U.S. an estimated 2500 cases of *L. monocytogenes* and as many as 500 deaths per year has been reported by the Center for Disease Control and Prevention (CDC, 2001). It occurs in several well-defined populations particularly pregnant women, newborn, persons with weak immune systems, persons with cancer, diabetes, kidney diseases, and persons on medications, elderly and people suffering from AIDS. Persons who are suffering from AIDS are 300 times more likely to encounter listeriosis than normal adults (CDC, 2001). Active surveillance of sepsis and meningitis by CDC (2000) shows that attack rates of listeriosis are 0.7 cases /100,000 population, 10 cases /100,000 in infants and 1.4 cases /100,000 in elderly persons. The probability and risk of developing the disease depends on the host susceptibility, degree, amount of bacteria ingested and the virulence of the strain.

The signs of an acute infection are superficial and accelerated respiration, slight cyanosis, lethargy, fever and anorexia. Convulsions, muscular twitching, sudden stop of respiration accompanied with severe cyanosis, increased irritability, tension, meningismus indicate infection of the central nervous system (CNS).

Listeric infection can be grouped based on the most prominent clinical symptoms as:

a) Septicaemic form, in which symptoms seen are an increase in the number of monocytes, sore throat and meningitis. *Listeria* affects the meninges, brain and the medulla with symptoms like purulent meningitis, encephalitis, and brain abscission. During meningitis the person experiences neck rigidity, nausea, vomiting and photophobia. The symptoms of neurolisteriosis adults are disturbances in language (caused by inhalation of infected dust particles), walking, loss of memory and some distinct Parkinson's disease (Seeliger,1961).

b) Oculo-glandular form, in this *Listeria* acts as a secondary invader and complicates latent tuberculosis of the cervical lymph nodes. Conjunctivitis is seen with symptoms like enlargement of parotid, fever and lymphocytosis ((Ryser et al., 1991c).

2.4.2. Listeriosis in pregnant women and newborn:

Hormonal changes during pregnancy have an effect on the mother's immune system. Pregnant women are 20 times more susceptible than normal healthy adults and they account for $1/3^{rd}$ of cases due to listeriosis. Mothers carrying *listeria* can contaminate the skin and respiratory tract of their babies during birth. The newborn develop symptoms after 2-3 weeks of exposure. (USDA, 2001). Infection of the fetus during pregnancy can be fatal and lead to either abortion or death of the newborn. Some of the symptoms during the weeks before abortion are acute fever, chills, headaches, diarrhea and backaches. This occurs when women become carriers of *L. monocytogenes* for

a considerable period of time. The organism is contained in the reproductive organs and this endangers the fetus. After recovery the mother can still serve as a carrier for a considerable period and lead to recurring problems during later pregnancies. (Seeliger, 1961). If the pregnant women are tested for listeriosis much before delivery, then there are chances for treatment *in utero* but if left untreated the infant remains critically ill (Schlech,. 2000).

In the case of newborns, infection can be via transplacental route or by the aspiration of amniotic fluid containing *Listeria*. If the infection of the fetus occurs during or before 4-5 months of pregnancy, then abortion of the fetus is commonly seen. This is called as 'early-onset' Listeriosis and results in abortion, stillbirth, or premature delivery of a severely affected infant. Mortality during this stage is ~20% while the chances of abortion and stillbirth increases mortality rate to >50%. If the infection occurs during later stages of gestation, the infant is born healthy and shows disease symptoms after 7-20 days of delivery. The newborn shows physical signs of meningeal irritation. This is called as 'late-onset' listeriosis and the mortality rate is ~10% (Schlech,. 2000).

2.4.3. Treatment for Listeriosis:

The time required for treatment may vary from 2 weeks for cases involving uncomplicated sepsis to 4-6 weeks for severe conditions like endocarditis in adults. Antibiotic therapy is required to prevent disabilities and death. Antibiotics like penicillin, ampicillin, tetracycline, erythromycin, rifampin, chloramphenicol and cephalothin are used since *L. monocytogenes* is sensitive to them. Tetracycline was not recommended due to side effects like staining of teeth and

altering of bone development in children. Use of chloramphenicol or streptomycin is also limited because of their toxic nature to neonates (Ryser et al., 1991c). Studies conducted by Schlech (2000) showed that listeriosis could be treated effectively using a combination of ampicillin and an aminoglycoside like gentamicin. Vancomycin in combination with an aminoglycoside was also successful in treating the infection for people who are allergic to penicillin. In general the minimum concentration of these antibiotics needed to kill (bactericidal action) the organism is many times higher than the levels needed to prevent the growth of the organism. Persons suffering with listeriosis are generally given a dose that is enough to prevent growth rather than to eliminate the organism. Adults suffering with septicemic forms of *listeria* generally receive 6-12 gm of ampicillin (i.v.) daily in 3-4 doses, newborns are given 200-400 mg/kg body weight/ day and pregnant women are given 3-6 gm/ day for 2-3 weeks. Persons suffering with oculoglandular or cutaneous forms of listeriosis should be given 3-6 gm of ampicillin daily until the symptoms disappear (Ryser et al., 1991c). Therefore even though the risk of listeriosis is relatively low in healthy adults, effective methods in the production, processing, handling and storage of foods is required to prevent devastating consequences.

2.5. Foodborne outbreaks due to *L. monocytogenes*:

The first documented foodborne outbreak occurred in Halle, East Germany between 1949-1957 and was attributed to the consumption of raw, unpasteurized milk (Ryser et al., 1991c). Out of 50 published investigations by FDA in 2000, 28.9% of outbreaks occurred in the U.S. while the rest occurred

outside the U.S. Out of the outbreaks occurred, food was identified as a vehicle in 13.3% of outbreaks and 59.5% of the outbreaks in the U.S. and outside the U.S., respectively (FDA, 2001).

2.5.1. Outbreaks in the U.S. and Canada:

The first foodborne outbreak of listeriosis was observed in 1979, where at least 23 patients were hospitalized in Boston, MA. Epidemiological studies linked hospital food as the vehicle of contamination. The food consumed contained lettuce, carrots and radish (Donnelly, 2001). In 1981 in Nova Scotia, Canada an outbreak occurred involving 41 cases, resulting in 17 deaths due to the consumption of contaminated coleslaw. Coleslaw obtained from the refrigerator of a patient indicated that it was prepared with cabbage and carrots and was purchased from a local market. Investigation of the source of cabbage indicated that it was obtained from a farmer who raised cabbage along with a flock of sheep infected with *L. monocytogenes*. Use of manure from infected sheep followed by cold storage of the cabbage was suspected as the factors causing the outbreak (Beuchat, 1995). The next outbreak occurred in Boston in 1983 involving 43 cases in which post-process milk was incriminated. This was followed by an outbreak involving 142 cases and 40 deaths in 1985 due to the consumption of Mexican-style cheese in California.

Surveillance by the FDA have resulted in numerous recalls of products like cheese, ice cream, and other dairy products (Kozak et al., 1996). A review of the records of the plant showed a 10% more delivery of milk than the pasteurizer could pasteurize and thus leaving a portion of milk unpasteurized (Hird, 1987). In

1994 an outbreak linked to consumption of chocolate milk involving 45 cases occurred in Illinois. Post-processing contamination of the milk was implicated as the vehicle (Donnelly, 2001).

During August 1998 to March 1999, 101 cases of listeriosis, including 21deaths was reported due to the consumption of hot dogs manufactured in Michigan. The hypothesis for the outbreak was cross-contamination of the product from food contact surfaces contaminated with *L. monocytogenes* (Mazzotta et al., 2001). Another reported outbreak due to acute febrile gastroenteritis occurred in LosAngeles due to the consumption of *L. monocytogenes* contaminated delicatessen meat effecting 44 people (Frye et al., 2002). Finally, a multistate outbreak effecting 52 people occurred in 2002 due to the consumption of contaminated sliced turkey (MMWR, 2002).

2.5.2. Efforts to control *L. monocytogenes*:

Numerous outbreaks and product recalls due to contamination of RTE meats with *L. monocytogenes* occurred during 1980's and 1990's. The latest figures from the Centers for Disease Control and Prevention (CDC) indicate that there have been as many as 2500 cases and 500 deaths per year due to listeriosis (CDC, 2002). As a result, U.S. Federal regulatory agencies and the food industry embarked on a number of initiatives to control this deadly pathogen. In response, the FDA developed the Dairy Safety Initiatives Program in 1986, and the USDA monitoring program for *L. monocytogenes* in meat products was developed in 1987. According to the USDA any RTE food that contained *L. monocytogenes* can be considered as adultered and is subjected to

a Class I recall (FSIS, 2002). Alarmingly the RTE meat sampling program by the USDA-FSIS from 1998-2001 showed the incidence rate of *L. monocytogenes* as 5.7% in sliced ham/luncheon meat, 4.4% in hotdogs, 3.1% in cooked roast beef and 2.4% in cooked poultry. Since the organism has the ability to grow under refrigerated conditions, the presence of even a low inoculum in foods during manufacturing can be harmful to consumers. Because of the potential hazards to consumers, the USDA has issued a zero-tolerance policy for this organism in RTE foods (Fred et al., 1996).

The Canadian policies direct all inspection and compliance actions towards products that can support growth of *L. monocytogenes*. In contrast to the U.S. approach, the Canadian regulatory agencies divide RTE foods divided into 3 risk groups (Table 2.).

Table 2. Canadian compliance criteria for *L. monocytogenes* in RTE foods.

Category	Action level for *L.monocytogenes*	GMP status	Immediate action	Follow-up action
I. RTE foods casually linked to listeriosis. This list presently includes: soft cheese, liver pate, coleslaw mix with shelf-life>10days, jelled pork tongue[a].	>0 CFU/50g[c]	N/A[a]	Class I recall to retail level. Consideration of public alert. Appropriate follow-up at the plant level.	
II. All other RTE foods supporting growth of L.monocytogenes with refrigerated shelf-life >10days.	>0 CFU/50g[c]	N/A	Class II recall to retail level. Consideration of public alert. Appropriate follow-up at the plant level.	

III. RTE foods supporting growth of *L.monocytogenes* with refrigerated shelf-life <10 days and all RTE foods not supporting growth[b]	<100 CFU/50g[c]	Adequate GMP	Allow sale	Appropriate follow-up at plant level.
	<100 cfu/50g[d]	Inadequate or no GMP[f]	Consideration of class II recall or stop sale.	Appropriate follow-up at plant level.
	>100 CFU/g[d]	N/A	Class II recall or stop sale.	Appropriate follow-up at plant level.

(Farber et al.,1996).

[a]At present,this product is not commonly found in the Canadian marketplace.
[b]RTE foods not supporting growth of *L. monocytogenes* includes the following:
- pH 5.0-5.5 and a_w <0.95.
- pH <5.0 regardless of a_w.
- a_w <0.92 regardless of pH.
- Frozen foods.

[c]Enumeration by enrichment only
[d]Enumeration done by direct plating onto selective agar.
[e]N/A not applicable
[f]No information on GMP is considered as no GMP. Burden of proof remains with the legal agent.

2.6. Recalls of RTE products due to *L. monocytogenes* in the U.S:

A large number of product recalls has resulted due to contamination of RTE foods with *L. monocytogenes* detected during the microbiological sampling program by FSIS. Some of them are listed in Table 3.

Table 3. Class I recalls of different meat products due to contamination with *L. monocytogenes* provided by FSIS.

Product	Date	Origin	Quantity (lbs)	Company	Reference
Fresh & frozen turkey, chicken breast	11/20/2002	New Jersey	4.2 million	Jack Lambersky poultry company Inc.	FSIS, 2002a
Fresh & frozen turkey and chicken	10/12/2002	Franconia, Pennsylvania	27.4million	Wampler foods, Piligrim's Pride Corp.	FSIS, 2002b
Franks & hotdogs	4/25/2002	Ohio	140,000	John Morrell & Co.	FSIS, 2002c
Fully cooked turkey	3/15/2002	Minnesota	23,000	West Central Turkeys Inc.	FSIS, 2002d
Luncheon	10/31/2001	Alabama	189,000	R.L. Zeigler	FSIS, 2001a

meat				Co.	
Luncheon meats, hams, sausages, hotdogs	4/12/2001	Oklahoma	14.5million	Bar-S foods	FSIS, 2001b
Poultry products luncheon meats	12/14/2000	Waco, Texas	18million	Cargill foods	FSIS, 2000.
Hotdogs & luncheon meats	1/28/1999	Zeeland, Michigan	35million	Bil Mar	FSIS, 1999a
Frankfurter & luncheon meats	1/22/1999	Arkansas	30million	Thron Apple Valley	FSIS, 1999b
Lunch meat products	11/4/1998	Florida	192,553	Dixie Packers Inc.	FSIS, 1998

2.7. Incidence of *L. monocytogenes* on food processing equipment:

Listeriosis can be prevented by controlling the incidence of the organism in the food-processing environment and by proper attention to the type of food material and its method of preparation. Due to the ubiquitous nature of the organism, it is readily introduced into the abattoirs and the meat processing environment. This along with its unique properties makes the organism difficult to eliminate completely. Meticulous attention to principles of industrial hygiene like HACCP can reduce the incidence of the pathogen (Schlech, 2000). Accordingly the USDA, FSIS and FDA provides guidelines to people of all risk groups, these include avoiding of hot dogs, luncheon meats or deli meat unless they are reheated, avoiding soft cheeses like feta, brie camembert, blue-veined and Mexican-style cheese (USDA, 2001).

2.7.1. Sources of entry:

Recontamination of RTE products was the primary source of *L. monocytogenes* in many processing environments. The source of the pathogen

was limited to certain specific sites of harborage including slicers (in sliced lunch meats), bagging tables (large cooked products), brine chill tunnel (hams), chilling and cutting rooms (pork) and between freezer and packaging machine (cooked meat patties) (Tompkin, 2002). Contamination during peeling just prior to packaging was the major source of entry of pathogen in the preparation of turkey franks (Wenger et al., 1990). Chasseignaux et al. (2002) showed that a surface made with resin or plastic that is uneven with organic residues, neutral pH, low temperature and high hygrometry was associated with contamination by *L. monocytogenes*. The organism is also detected on the floors, walls, hand basins, splitting saws, and chopping blocks in the meat processing environment (Borch et al., 2002). Studies on the prevalence of *L. monocytogenes* show that 16% of raw pork meat and 17% of poultry meats are contaminated with *L. monocytogenes*.

About 8% of samples in poultry slaughterhouses, 26% of samples in raw poultry meat plants, and 68% of samples in raw pork and meat plants were contaminated with *L. monocytogenes* (Chasseignaux et al., 2002). Despite best efforts, complete elimination of *L. monocytogenes* may be nearly impossible. However, by maintaining clean, dry floors and by paying diligent attention to GMP, which include plans for rigorous cleaning and sanitizing programs for equipment, incidence of *L. monocytogenes* can be controlled (Tompkin, 2002). Careful attention to the movement of traffic from raw product locations to final product areas is required to prevent contamination (Kozak et al., 1996).

2.7.2. Control measures:

Commercial sanitizers and cleaning compounds are used to control the spread of *L. monocytogenes* in processing facilities. These include the use of compounds like quaternary ammonium compounds (QAC, 800-1000 ppm), sodium hypochlorite, iodophors, and acid sanitizers. All of these compounds exhibit bactericidal activity and reduce the bacterial population by 4-5 $\log_{10}$ during first 30 sec. The possible mode of action being, diffusing into the cell, formation of toxic compounds which intum inhibit key enzymatic reactions and alter cell membrane permeability (Ryser et al., 1991d). Studies conducted by Mustapha et al. (1988) showed that 200 ppm and 400 ppm of sodium hypochlorite applied for 2 min was efficient for the destruction of *L. monocytogenes* on smooth and porous surfaces, respectively. They also concluded that 50 ppm of QAC applied for 2min was sufficient to obtain >4 $\log_{10}$ reduction in cell numbers.

Lee et al. (1991) studied the effect of sodium hypochlorite on *L. monocytogenes* attached to stainless steel surfaces. The results indicated that using 50 ppm chlorine followed by a 30 sec heat treatment at 65°C reduced the cell numbers to undetectable levels and concluded that a 5 min exposure to 200 ppm chlorine solution is required for *L. monocytogenes* inactivation on stainless steel surfaces. Peroxyacetic acid and glutaraldehyde were effective in controlling *L. monocytogenes* in milk and meat processing facilities. Since water-based chain conveyor lubricants also serve as a potential source for *L. monocytogenes*, sanitizing agents can be included into lubricants to minimize spread of the organism (Ryser et al., 1991d). Research is also being conducted on the use of

antimicrobials of natural origin (nisin, lysozyme) to inhibit adhesion of pathogens like *L. monocytogenes* to food contact surfaces (USDA, 1999).

2.8 Microbial Interventions:

As a result of market globalization and a need to meet the consumer needs for high quality and convenient meat products with natural flavor and taste, food manufacturers employ a variety of processing techniques (Hugas et al., 2002). The most common methods of meat preservation include the application of heat, pressure, acid treatment, salts of organic acids, irradiation, and chemicals. These reduce the potential pathogens in foods by creating conditions that are unfavorable for growth and survival. All these methods aim to control the pathogen while having a mild effect on the food. They also offer a wide range of applications by various combinations to control *L. monocytogenes*.

2.8.1. High-pressure pasteurization:

High pressure processing (HPP) represents a promising and attractive non-thermal process for the preservation of sliced cooked cured meat products. High-pressure technology between 100-600 Mpa is of increasing interest to biological and food safety systems to control microbial growth at low or moderate temperature without affecting the organoleptic properties of meats. The level of inactivation by HPP depends on the type of microorganism, level of pressure, time of treatment, temperature, pH, water activity and the composition of foods (Hugas et al., 2002). Although research involving the application of pressure to meat is limited, several studies have been conducted on the application of pressure to control *L. monocytogenes* in broth/growth media or emulsions and

food suspensions using *L. innocua* as a model. (*L. innocua* is a nonpathogenic species of *Listeria* with heat resistance similar to that of *L. monocytogenes*).

Lanciotti et al. (1994) studied the effect of 100 Mpa on a suspension of *L. monocytogenes* and reported that a high-pressure homogenization treatment around 100 Mpa causes a reduction in *L. monocytogenes*. Similarly Wuytack et al. (2002) studied the effects on *L. innocua* suspensions and obtained 2 $\log_{10}$ reduction at 300 Mpa and concluded that >4 $\log_{10}$ reduction can be obtained after 4 rounds of homogenization. Cheftel et al. (1997) studied pressure resistance of *L. monocytogenes* by applying pressure on minced beef for 20 min at 20°C using *L. innocua* as a model. They observed a 5 $\log_{10}$ reduction at pressure above 400Mpa. Higher (35°C) or lower temperature (4°C) enhanced the inactivation.

Cell death can be due to multiple or accumulated damage inside the cell, the cell wall often is the main target. Cellular functions sensitive to pressure include modification of membrane permeability, fatty acid composition, cell and membrane morphology, protein denaturation, inhibition of enzyme activity, formation of vacuoles etc. Thus change in membrane structure is the main factor of inactivation (Lado et al., 2002). Occurrence of bud scars on the surface of cells altering the membrane morphology was observed when 400 Mpa pressure was applied to buffer containing *L. monocytogenes* for 10 min (Ritz et al., 2002).

A synergistic effect of antimicrobial compounds with HPP in a meat model system was conducted by Hugas et al. (2002). They obtained $<10^2$ CFU/g of *L. monocytogenes* until 16days of storage when nisin was used after application of high pressure (400 Mpa, 10 min, 17°C). When a meat model system containing

cooked ham was treated with sakacin, enterocins, and pediocin after a pressure treatment of 400 Mpa for 10 min, the population of *L. monocytogenes* was kept below 10^2 CFU/g (6 $\log_{10}$ reduction) until the end of storage (61 days, 4°C). (Garriga et al., 2002). A 10 $\log_{10}$ reduction of *L. monocytogenes* in ground beef patties using a mild heat treatment at 50°C simultaneously with HPP at 414 Mpa for 6 min was observed by Murano et al. (1999). The application of pressure has advantages as well as disadvantages. Stressed cells can develop resistance and exhibit growth during storage, which is not desirable. Also stressed cells may be less heat resistant, so a combination of pressure and heat may lead to excellent reduction of the pathogen (Cheftel et al., 1997).

2.8.2. Irradiation:

Irradiation also known, as cold pasteurization, is an effective control measure in maintaining the quality of raw, cooked and minimally processed meat products (Molins et al., 2001). The FDA/WHO Codex Alimentarious Commission consider irradiation as a safe technology for controlling *L. monocytogenes* in raw and uncooked meat. Among the different forms of irradiation UV, gamma radiation and electron beam (using electricity) are considered to be bactericidal.

UV rays because of their poor penetration power are restricted to the treatment of food and non-food contact surfaces and eradication of airborne contaminants. Studies involving UV radiation to meat and *L. monocytogenes* is limited. Collins et al. (1971) determined the susceptibility of *L. monocytogenes* to UV rays in Tryptic Soy Agar plates by exposing to a radiation output of 40W/cm^2 for 30, 60, 90 sec and found that the D-value for *L. monocytogenes* was 60 sec.

The rate of inactivation increased with time. UV radiation can thus be of some practical use in reducing *L. monocytogenes* in food production and storage area. By the year 2001, 40 countries have permitted use of irradiation in many different types of foods including 12 countries that use irradiation for control of pathogen in poultry, 8 countries permit for use in meat and 13 in fish and seafoods (Molins et al., 2001).

Gamma radiation:

Gamma radiation, having excellent penetration power makes it suitable to effectively control *L. monocytogenes* in uncooked meats, but has not been yet approved for use on RTE meats. A petition to permit its application on RTE foods is under review by FDA (Clardy et al., 2002). Currently a dosage level as high as 10 KGy is permitted for use to control pathogens in foods (Aziz et al., 2002). Clardy et al. (2002) found that a dose of 3.9 KGy reduced *L. monocytogenes* populations by 5 $\log_{10}$ units in frozen ham sandwiches. Thayer et al. (1995) found that the D-values for *L. monocytogenes* in uncooked beef, lamb, pork and turkey stored at 5°C ranged from 0.45-0.5 KGy.

Monk et al. (1994) applied a dose of 2.5 KGy to raw ground beef patties and observed 4.1 $\log_{10}$ reduction in the population of *L. monocytogenes*. They also observed that neither the fat content nor the temperature during treatment effected the inactivation rate. Fu et al. (1995) applied a radiation dose of 0.9 KGy on ham inoculated with *L. monocytogenes* and obtained a 3 $\log_{10}$ reduction. They also observed that a dose of 2.0 KGy at 7-10°C reduced the population of *L. monocytogenes* to undetectable levels. Gamma radiation (2.9 KGy) in

combination with heat (65.6°C) was applied to chicken breast by Shamsuzzaman et al. (1992) and they observed that *L. monocytogenes* was reduced to undetectable levels for 8 weeks. Sommers et al. (2002) found that a dose of 2.3-3.1 Kgy was required to obtain a 5 $\log_{10}$ reduction in *L. monocytogenes* on vacuum packaged cooked beef bologna containing dextrose. They also observed certain undesirable changes in meat these include acceleration of lipid oxidation, breakdown of PUFA and formation of MDA and also loss of red color. Some studies indicate that organic acids like citric acid enhance the bactericidal effects of ionizing radiation (Sommers et al. 2003). Although effective, irradiation affects the color of raw and cured meats. Sensory panelists could differentiate between irradiated and non-irradiated products. Irradiated foods also suffer from a negative public image because of its chances of having carcinogenic effects (Stermer et al., 1987).

2.8.3. Acid treatment:

Since emerging technologies like irradiation are not permitted for use on RTE foods, there has been renewed interest in the application of GRAS chemicals and organic acids as hurdles. Acidification of foods is an age-old method of preservation and can be used to create an adverse environment to the growth of *L. monocytogenes*. The type of acid, pH, temperature and other antimicrobial compounds all play an important to create an effective barrier against the pathogen (Doyle et al., 1999). The antimicrobial action of an acid depends on the extent of undissociation. Weak acids like acetic acid can diffuse into the cell, lower the intracellular pH resulting in the inhibition of certain

metabolic and anabolic processes (Abee et al., 1999). Rinsing of the carcass with acetic acid is permitted in the U.S. (FSIS, 1992). Samelis et al. (2001) observed the effect of dipping *L. monocytogenes*-inoculated sliced bologna for 1 min in 2.5% and 5.0% of acetic and lactic acid solutions before vacuum packaging and stored at 4°C. At 5.0% acetic acid they found an inhibition of the pathogen for 120 days while 5.0% lactic acid inhibited growth for 50-80 days before significant growth occurred. Using 2.5% acetic acid was antimicrobial until 70days while 2.5% lactic acid permitted significant growth within 20-35 days. They also found that inoculation before dipping didn't differ in effect when compared to dipping after inoculation.

A post-heat processing, organic acid dip treatment of pork frankfurters to control *L. monocytogenes* was evaluated by Palumbo et al (1994). They used a 2 min dip in a mixture of acetic acid and citric acid (at 2.5% each), 5% acetic acid, or 5% lactic acid and observed that the combination treatment was effective in restricting growth for 90 days when stored at 5°C while the individual acids also suppressed the pathogen during the 90 days storage period. When lean pork fat and tissue that were artificially inoculated with *L. monocytogenes* were dipped for 15 sec in 3% lactic acid at 55°C, a 2-3 $\log_{10}$ reduction was observed and the bacterial population remained unchanged following the 15 days storage period (Green et al., 1995). Podolak et al. (1996) found that fumaric acid at a concentration of 1% was effective in reducing the population of *L. monocytogenes* by 1 $\log_{10}$ on artificially contaminated raw beef. A treatment involving 2% polylactic acid, 2% lactic acid, 400 IU/ml nisin, or a combination of

each acid and nisin for 5 min was shown to reduce the population of *L. monocytogenes* within the range of 0.97-1.97 $\log_{10}$ cycle on vacuum-packed fresh beef stored at 4°C for 42 days. Addition of organic acids increased the effectiveness of nisin to reduce *L. monocytogenes* (Ariyapitipun et al., 1999). Acetic acid was found to be more inhibitory than lactic acid because of its ability to diffuse through the cell membrane and deposit in the membrane and finally effect substrate transport (Vasseur et al., 1999). Thus either one used alone, or in combination, with other organic acids have the potential of reducing *L. monocytogenes* in meat products.

2.8.4. Salts of organic acids:

One of the emerging food safety tools to combat *L. monocytogenes* in RTE meats is use of antimicrobial substances like sodium or potassium lactate, sodium nitrite, sodium propionate, sodium diacetate, potassium sorbate and sodium benzoate. These substances when used either singly or in combination reduce the risk of foodborne illness due to *L. monocytogenes*. Sodium diacetate was registered by EPA in 1968 as a food preservative and is considered a GRAS chemical. CFR part 424 deals with the application of these chemicals as a food additive in meat and poultry foods. FSIS permits the use of sodium diacetate at a level of 0.25% in meat and poultry products in accordance to 21 CFR 184.1754 (EPA 1991, FSIS 1999).

Combination of low a_w and pH are important for the inhibitory effects of the salts towards *L. monocytogenes* (Ryser et al., 1991d). The current permitted levels of sodium lactate and sodium diacetate are 4.8% and 0.25%, respectively.

(Glass et al., 1999). A study conducted by Mbandi et al., (2002) evaluated the effect of sodium lactate (2.5%), sodium diacetate (0.2%) and their combination on a single strain (*L. monocytogenes* Scott A) and a mixture of 6 strains of *L. monocytogenes* inoculated beef bologna and then stored at 5°C and 10°C. They found that each of the salts alone delayed the growth of *L. monocytogenes* for about 30days while the salt combination inhibited growth at both 5°C and 10°C. Bedie et al. (2001) studied the effect of current permissible and increased levels of sodium lactate (3% or 6%), sodium acetate (0.25% or 5.0%) and sodium diacetate (0.25% or 5.0%) included in frankfurter formulation stored at 4°C on *L. monocytogenes*. The results obtained indicate that sodium lactate (3%) was more effective in controlling *L. monocytogenes* than sodium diacetate (0.25%), which was better than sodium acetate (0.25%) for 70, 35 and 20 days respectively. At levels of sodium lactate (6%) and sodium diacetate (0.5%) higher than that currently permitted complete inhibition of *L. monocytogenes* was found for 120days on frankfurters stored at 4°C. A study was conducted to examine the efficacy of lactic acid (0.5%) and sodium benzoate (0.05%) to reduce *L. monocytogenes* on raw chicken stored at 4°C for 0, 2, 4, 6, 8 days. Cheng-an et al. (1995) found that a solution containing the above solutions can reduce the population of *L. monocytogenes* until 8 days when stored at 4°C. Islam et al. (2002) studied the effect of 4 different preservatives (sodium benzoate, sodium propionate, potassium sorbate and sodium diacetate) by dipping *L.monocytogenes*-inoculated turkey frankfurters for 1 min in these solutions. The solutions were prepared to 15, 20 or 25% wt/vol with <0.3% of the

chemical in the frankfurter. The results revealed that the *L. monocytogenes* population decreased by 1-2 $\log_{10}$ immediately and storage at 4°C for 14 days showed 3-4 $\log_{10}$ reduction for all treatment concentrations. Storage at 13°C for 14days showed a 3.5-4.5 $\log_{10}$ reduction for treatments using 25% sodium benzoate or 25% sodium diacetate and a 2.5 $\log_{10}$ reduction for treatments involving 25% sodium propionate or 25% potassium sorbate. Only the treatments involving 25% sodium diacetate or sodium benzoate showed significant inhibition of *L. monocytogenes* on frankfurters stored at 22°C for 7 days or longer.

Blom et al. (1997) studied the effect of a mixture of 2.5% lactate and 0.25% acetate (w/w) on *L. monocytogenes*-inoculated sliced servelat sausage and cooked ham, which was then stored at 4°C. They found that population of *L. monocytogenes* was inhibited for a storage period of 5 weeks in both servelat and cooked ham stored at 4°C and for 2-3 weeks at 9°C. Miller et al. (1994) found that 3% and 4% sodium lactate had inhibitory effects on beef top rounds inoculated with *L. monocytogenes* and stored at 10°C for 28 days. In all these studies sensory analysis was conducted to evaluate appearance, flavor and overall satisfaction and the results revealed that no significant difference existed between treated and untreated samples.

2.8.5. Thermal Inactivation:

Thermal processing for the inactivation of microorganisms is most widely used and is the fundamental method used to preserve food and for providing safer food for human consumption (Ryser et al., 1991d). Pasteurization of raw

milk has been widely used and is considered as a safe process to reduce *L. monocytogenes* to levels that don't pose a risk to human health. High temperature short time (HTST) pasteurization (71.6°C/15 sec) was found to be sufficient to destroy *L. monocytogenes* in milk (Mathew et al. 2002; Ryser et al., 1991). Ever since pasteurization was found to be effective to control *L. monocytogenes* in milk, there has been heightened interest to apply heat to *L. monocytogenes* in meats. Cooksey et al. (1993) applied post-package pasteurization followed by refrigeration storage to control pathogens as well as surface contamination on RTE precooked beef. Pasteurization at 82°C for 16 min increased the shelf-life upto 85 days when stored at 4°C. Cooksey et al. (1993) then applied post-package pasteurization to control *L. monocytogenes* in precooked vacuum-packaged beef loin chunks. They observed that pasteurization effectively eliminated *L. monocytogenes* and reduced the levels by 10,000 fold on the surface and by 1,000,000 fold in broth. The population of *L. monocytogenes* remained unchanged during 85 days of refrigerated storage. Hardin et al. (1993) examined the fate of *L. monocytogenes*-inoculated precooked beef roasts subjected to various post-package treatments and also evaluated the storage stability at 4, 8 and12 days (at 4°C) and for 8, 14, 28 and 56 days (at 10°C). The process subjected the inoculated vacuum-packaged beef roast to hot water bath pasteurization at 2 different temperatures (196°C and 205°C) and for 2 different dwell times (3 or 5 min), thus a total of 4 different treatments were tested. They found that treatment involving 5 min at 196°C showed the greatest decrease (4.5 $\log_{10}$ reduction) in *L. monocytogenes*

population on day 1 and further decreased throughout storage at both 4°C and 10°C. Several studies have been done using *L. innocua* as a heat resistant model for *L. monocytogenes*. It is considered to be nonpathogenic and having heat resistance similar to, or greater, than *L. monocytogenes*.

Vacuum/steam/vacuum surface pasteurization was applied to control *L. innocua* inoculated hotdogs. One cycle involved an initial vacuum for 0.1 sec, two cycles with 0.3 sec of steam exposure with a final and inter-cycle vacuum period of 0.3 sec. For a total process time of1.3 sec, Kozempel et al. (2000) obtained >3 $\log_{10}$ reduction. Pasteurization for 3 or 4 cycles can further reduce the population of *L. innocua* by 5 $\log_{10}$ cycles. Murphy et al. (2002) achieved a 7 $\log_{10}$ reduction of *L. innocua* by using post package treatment of fully cooked chicken breast strips. The treatment involved 20 min (for a 227 gm package) or 34 min (for 454 gm package) process time in a hot water cooker at 88°C. Cygnarowicz-provost et al. (1994) applied flash steam pasteurization for 32 sec at 136°C to *L. monocytogenes*-inoculated beef frankfurters and obtained a 4 $\log_{10}$ reduction. They found that levels remained reduced when stored at 6°C and 19°C without any difference in color and weight between treated and untreated samples.

Bersot et al. (2001) obtained a 3 $\log_{10}$ reduction of *L. monocytogenes* in RTE mortadella ham that was cooked to an internal temperature of 74°C in the coldest point and the population remained low for 30 days when stored at 7°C. Chikthimmah et al. (2001) obtained a 5 $\log_{10}$ reduction of *L. monocytogenes* during a commercial Lebanon bologna manufacturing process by fermenting the

raw mix to pH 5.0 at 37.7°C and then heating to 46.1°C for 5 hrs. Muriana et al. (2002) applied post-packaged submersed water pasteurization for reducing *L. monocytogenes* on different RTE products and found that a processing time of 2 min at 195°C-205°C can readily provide a 2 $\log_{10}$ reduction. They were also able to achieve a 4 $\log_{10}$ reduction when the process time was extended to 4, 6, 8, or 10 min.

The objective of this study was to apply pre-package surface pasteurization using a radiant heat oven alone and in combination with post-package pasteurization to provide a potential hurdle effect on *L. monocytogenes* inoculated on RTE meats (bologna, deli ham, roast beef and turkey).

CHAPTER III

MATERIALS AND METHOD

3.1. Bacterial Strains:

A four-strain mixture of *L. monocytogenes* (Scott A-2, serotype 4b; V7-2, serotype 1/2a; 39-2 retail hotdog isolate; 383-2 ground beef isolate) was used for inoculation trials. These strains were made resistant to streptomycin (100 µg/ml; Sigma Chemical Co., St. Louis, MO) and rifamycin S/V (10 µg/ml; Sigma) and were plated on general purpose agar (Tryptic Soy Agar, TSA; Difco™, Becton-Dickenson, Franklin Lakes, NJ) containing these antibiotics when selectively plating for the inoculum cultures. This approach allows the recovery of viable and heat-injured cells without the need for harsh selective media that may prevent the growth of heat-injured cells (i.e., MOX agar) or in lieu of indigenous contaminating bacteria. The bacterial strains were cultured by transfer of 100 µl of thawed frozen culture into 10 ml of Brain Heart Infusion (BHI) broth and incubated overnight at 30°C; each of the four cultures were then transferred individually to 40 ml BHI culture and later combined (i.e., 160 ml) prior to use in the 'dip' inoculation treatment. For surface 'contact' inoculation, overnight cultures were mixed in equal proportions and the mixture was surface plated (100 µl) onto Tryptic Soy Agar (TSA) that was held overnight at 30°C.

3.2. Product Inoculation:

Product samples were generally 4-13 lbs (1.8-5.9 kg) that included roast beef (whole and split rounds), corned beef (whole logs), and ham (formed and whole muscle), except for turkey bologna (~2-lb sections). Except for 2 lots of

roast beef (received frozen and allowed to thaw) all products were received fresh/refrigerated from commercial processors as they would normally be shipped for sale to retailers without the additional thermal processing. The products were stored at 3°C (37.4°F) upon receipt and removed just prior to inoculation, so the internal temperature was the same. Immediately prior to use, products were taken from refrigerated storage, removed from their packaging wrap, and inoculated with *L. monocytogenes* by one of two different methods: a) dip inoculation method, or b) a contact inoculation method. Control product samples were also inoculated for each replication trial, but not heated, as they represented the basal recovery level for the inoculated microorganisms.

The 'dip' inoculation method: Approximately 160 ml of a 4-strain mixture (i.e., 4 x 40 ml) of *L. monocytogenes* was placed in a stainless steel bowl into which individual product pieces were 'dipped' by rotating product until all exposed surfaces were wetted with the mixed culture. Product pieces were then placed on a sterile tray for 5 min to drain off excess culture and then placed on a conveyor belt leading into the radiant heat oven. Using the dip inoculation method, we typically had inoculation levels of ~1-3 x 10^9 CFU per product as determined from recovery from inoculated, but unheated, control samples.

The 'contact' inoculation method: Sponge-foam padding material (~5-6 cm thick) was cut to the shape of a petri plate, autoclaved in foil-covered beakers, and used to pick up the mixed-strain inoculum lawn from inoculated petri plates after overnight incubation on agar plates using a contact and half-twist motion. The inoculum was then 'contact inoculated' onto the surface of the product using

the same half-twist motion. The inoculated product was then placed on the conveyor leading into the radiant heat oven. As determined from non-heated control samples, the contact inoculation method provided initial levels of 1-3 x 10^9 CFU per product sample.

3.3. Pre-package pasteurization with a radiant heat oven:

A radiant heat oven (commercial name is "Infrared Grill^{Tm}") was obtained from Unitherm Foodsystems (Bristow, OK) and installed (480 V, 30 Amp) in our Pathogen Processing Pilot Plant (Fig 9A-9B). The oven consisted of a stainless steel conveyor belt that passed through heating elements above and below the conveyor belt (Fig. 9). Heating coils had 12 in (30.5 cm) of lateral clearance at the level of the conveyor belt and 8 in (20.3 cm) of vertical clearance above the belt; a separate, bottom coil was positioned 5 in (12.7 cm) below the belt. The coils themselves were spaced at 2.5-3 in (6.3-7.6 cm) apart. Inoculated product pieces were passed through the radiant heat oven (Fig. 9) for various dwell times at full (#5 dial setting for ham and roast beef), or 80% power (#4 dial setting for turkey bologna). Products were processed at residence times of 45-120 sec by adjusting the speed of the belt and depending on the resilience of the product and the throughput requirements of the respective processors. Product logs were placed lengthwise on the belt. Half-rounds of roast beef were pasteurized both ways, with the cut face facing the end of the oven as well as to one side. After passage through the oven, product samples were transferred into a sterile bag, chilled in an ice-water slurry, and rinsed (massaged and shaken) with a chilled sterile diluent (50 mls of 0.1% buffered peptone water, BPW), to recover

cells for microbial analysis (usually within 15-20 min); inoculated, but unheated, control samples were treated similarly. The same procedure was repeated for different meat samples.

3.4. Post-package surface pasteurization:

Post-package surface pasteurization of fully-cooked deli ham, roast beef, and turkey bologna was performed using a 50-gal (189 liters) steam-injected temperature-controlled water bath similar to that used in earlier study by Muriana et al (2002). For samples processed by post-package pasteurization alone (roast beef), we used a 25 ml inoculum. Additional resuspension diluent (25-50 mls) was used after the pasteurization process to insure recovery of the remaining inoculum.

3.5. Combination pre- and post-package pasteurization:

A combination pasteurization process was examined that included a short pre-package pasteurization treatment (45 or 60 sec) of inoculated product followed quickly (<2 min) by vacuum packaging and post-package pasteurization (either 45, 60, or 90 sec) by submersion and subsequent microbial analysis as described previously (Muriana et al., 2002).

3.6. Product temperature measurement:

Product temperatures were examined by several methods. Temperature-hardened DataTrace^{Tm} probes (Mesa Labs, Lakewood, CO) were placed at the top, bottom (offset to one side), sides, front, and back of turkey bologna in order to examine the temperature distribution of the oven on all sides of product that were not easy to obtain by any other method (Fig. 9C). An infrared digital

thermometer (Raytek, Raynger model ST80, Santa Cruz, CA) that could provide the average/minimum/maximum temperature of 8 infrared dots in a circular pattern was also used (Fig. 9D).

3.7. Microbiological analysis:

After radiant heat and/or post-package pasteurization, the remaining inoculum bacteria were recovered by placing products into large sterile bags, into which were added 25-50 ml of BPW. The bags were then shaken and massaged for 5 mins to resuspend surviving bacteria into the rinse buffer. Recovery of the rinse buffer was followed by appropriate serial dilutions and was pour plated using TSA containing the antibiotics specified earlier. The plates were then incubated for 48 hrs at 30°C.

3.8. Experimental Design:

Except for one study with frozen/thawed roast beef that was done in duplicate, all trials were performed in triplicate replications. Inoculated control samples and experimental samples were run in pairs at each processing condition within a replicate. Different replications were done on separate days, with different lots of the same product, and with pairs of samples from the same lot at each test condition. Standard deviations were obtained for multiple samples in the various replications. Residence times were limited to those of practical application to the various participating processors.

CHAPTER IV
RESULTS AND DISCUSSION

Post-processing contamination of RTE meats with *L. monocytogenes* has become a major concern to the value-added processed meat industry and surface pasteurization is becoming an effective means in reducing risk from such products. Much of the work on "meat surface pasteurization" has been done in relation to carcass pasteurization (i.e., Frigoscandia, Inc.) that is intended to steam-pasteurize exposed raw beef carcass surfaces to reduce the incidence of *E. coli* O157:H7 that would end up in trimmings and possibly, ground beef (Gill and Bryant, 1997). The *Listeria* problems currently encountered in the RTE processed meat industry are a combination of a) the presence of environmental/worker *Listeria* contamination, and b) a high degree of post-process product exposure and handling that could possibly allow acquisition of incidental surface contamination (worker handling, removal of deli product from cook-in bags, exposed product on trays/carts wheeled into smoke houses, etc). In order to provide a solution to this problem, we have been studying the application of surface pasteurization as a convenient and effective means of reducing incidental surface contamination on product surfaces immediately before (pre-package) or after (post-package) final packaging. Post-package pasteurization (Muriana et al, 2002) has already been implemented by several large meat processors and product that was processed and entered into commerce withstood a plant-wide recall due to *L. monocytogenes*.

In this study we examined *pre*-package surface pasteurization of RTE meats using a radiant heat oven alone (Fig. 9A, 9B), and in combination with post-package pasteurization (Muriana et al., 2002), to reduce incidental *L. monocytogenes* contamination that could be acquired during post-process handling and packaging.

We examined surface temperatures using "hardened" temperature probes with a turkey bologna product. By using paired placements of probes (Fig. 9C) on turkey bologna (top/offset-bottom, left/right side, front/rear face) we were able to examine the temperatures at the various surfaces to test for major discrepencies. The largest discrepency was observed between the upper and lower product surface temperatures (Fig. 10A) as a result of 'shielding' of the bottom of the product by the stainless steel mesh conveyor belt and was alleviated (Fig. 10B) by a design modification. In order to alleviate this condition, the manufacturer suggested making a rotational adjustment of the heating coils underlying the conveyor belt (Fig. 10C) to a closer proximity to the belt and the overlying product's bottom surface (Fig. 10D). This modification resulted in a noticeable and significant improvement in the top and bottom heating profiles compared to what was observed previously. It should be noted that the 'bottom' probes were placed 'offcenter' and were not influenced by the temperature of the belt, which is a nominal 95-99°F (35-37°C) upon its return to the oven entrance since approximately 65-70% of the circuit of the circular belt is outside the oven; this is also observed with larger commercial systems.

Although we used metal-tipped probes for surface measurements, we recognized that these could be susceptible to errors. If the probes were placed 1-2 mm too deep (along the surface), they may be measuring more sub-surface temperature, and if placed too high, they may be influenced more by air temperature and therefore, extreme care was taken in their placements. Our intention was to identify if gross temperature differences existed with different products of various shapes/sizes. We expected size differences would position the product closer to or farther from the upper heating coils which is a problem with any fixed-distance radiant oven. A hand-held infrared thermometer was also used that gave the average temperature from 8 infrared 'dots' projected onto the surface of a product (Fig. 9D). At first, this appeared as perhaps a better means of obtaining accurate surface temperature measurements. However, temperature values would change as either conveyor belt or hand movement would change the position of the dots and the exact points that were being measured. It is conceivable that a mounted infrared temperature sensor/monitor could provide continuous monitoring of product as it is exiting the oven to give continuous real-time alert if targeted surface temperatures are not achieved (i.e., pointing at the product as it exits the oven perhaps through a hole in the exit housing in which the product crosses the path of the beam). Using the hand-held infrared monitor, we observed surface temperatures for ham in the ranges of 138°-162°F (30 sec dwell time; 59°-72°C), 147°-189°F (45 sec; 64°-87°C), 154°-209°F (60 sec; 68°-98°C), and 165°-215°F (75 sec; 74-102°C). Some cut meat surfaces (turkey bologna, roast beef half-rounds) showed somewhat lower

temperatures than other surfaces, either due to the cut flat side being offset from directly facing the heat source, or possibly due to cut sides also showing slight sweating (purge) during heating. Occasionally, surface temperatures as high as 250°F (121°C) would be observed, but would quickly change to lower temperatures as the product moved.

As with post-package pasteurization, care should be taken in developing microbial reduction/processing models based on surface temperatures without confirmatory inoculated studies. It is clear from various high surface temperature measurements we obtained that the accompanying microbial reduction was not in line with what would be expected by extrapolation from D-values for the inoculated pathogens (Muriana et al., 2002). Unlike heating to a specific internal temperature for fully-cooked products where everything from the center-on-outwards has reached the target temperature (or more), brief surface heating may not necessarily penetrate all the cuts, folds, and crevices that can be accessed by bacteria and therefore single-point, or even multi-point, temperature readings of the outermost surface may be of limited practical application.

In previous studies with post-package pasteurization, a fixed amount of inoculum was added to each of the products in vacuum packaging bags before being vacuum sealed (Muriana et al., 2002). This method of inoculation had to be modified for use with surface inoculation of a non-packaged product and therefore we examined both a dip- and contact-inoculation method and contemplated the practical difference between the two methods after using them in several pasteurization trials. RTE deli ham and roast beef half-rounds were

surface pasteurized using both the dip and contact inoculation methods (Fig. 11). The results showed that the contact inoculation method demonstrated 1-2 $\log_{10}$ greater reduction than the extreme 'dip' inoculation method. During radiant heat surface pasteurization of hams inoculated with *L. monocytogenes*, we obtained a 0.75-1.85 $\log_{10}$ reduction using the dip method and 2.7-3.9 $\log_{10}$ reduction with the contact inoculation method during processing between 45-75 sec (Fig. 11A). Similarly, with roast beef we achieved a 1.5-2.2 $\log_{10}$ reduction (dip inoculation) compared to a 2.5-3.8-$\log_{10}$ reduction (contact inoculation) when processed for 60-90 sec (Fig. 11B). The differences between the two inoculation methods are reasonably assumed to be due to the aggressive infiltration of small cracks, crevices, and folds by the dip method that protects some of the bacteria from the full heating regimen.

From our results with radiant heat pasteurization, it appears that this process can reduce incidental contamination that may have been acquired upstream during post-process handling. We propose that this process could be most effective if placed just prior to final packaging for processes where currently no such microbial interventions exist. However, there could still be concerns for contamination during the final packaging, although this would be minimized if packaged immediately while product was still hot. With this in mind, we further examined a combined pre- and post-package pasteurization step which would gain the benefit of direct surface heating of pre-package pasteurization with the added benefit of further pasteurization after final packaging while the surface is still warm (with no further exposure due to handling). Using formed ham and

turkey bologna, we obtained a 1.35- and a 1.53-$\log_{10}$ reduction of *L. monocytogenes*, respectively, using a 60-sec radiant heat surface pasteurization step with dip-inoculated product, our most aggressive inoculation method (Fig. 12). When the pre-package pasteurized ham was followed with either a 60- or 90-sec post-package pasteurization step at 200°F (93.3°C), we obtained a combined 3.17- or 3.91-$\log_{10}$ reduction, respectively (Fig. 12A). When turkey bologna was followed with either a 45- or 60-sec submersed water post-package pasteurization step, we obtained a 2.73- or 4.3-$\log_{10}$ reduction, respectively (Fig. 12B).

After examining both, the dip and contact inoculation methods, we feel that the contact inoculation method is more typical of how incidental contamination is acquired in plants (cross-contamination with contaminated food contact surfaces) and suggest this method is a more practical way to surface-inoculate large non-packaged deli meat products. It is important to note that the contact inoculation method does not undercut the safety of process evaluation as the typical sponge-delivered 'contact inoculum' for our deli products inoculated circa ~10^9 CFU of *L. monocytogenes* per product piece tested and all products were inoculated in this manner on several sides. There is no conceivable way that fully-cooked product could contact-acquire such high levels of *Listeria* as we have inoculated unless permissive growth conditions were allowed.

In an additional roast beef study using only contact inoculation, we examined the effect of radiant heat surface pasteurization with whole- and half-rounds of roast beef positioned in the oven with the cut side either facing forward

or to the side, and comparison of frozen/thawed roast beef product processed by radiant heat alone and in combination with post-package pasteurization (Fig. 13). Radiant heat pasteurization of fresh refrigerated roast beef, both whole and half-rounds regardless of position, as well as whole logs of corned beef, gave similar levels of 2.15-2.45-$\log_{10}$ reduction (Figs. 13A & 13B). However, radiant heat pasteurization of frozen and thawed roast beef provided lower levels of reduction (1.5-$\log_{10}$), presumably due to destruction of meat cells leading to increased 'juiciness' of the roast beef after thawing (Fig. 13B). When frozen/thawed roast beef was processed via short term post-package pasteurization (60 and 90 sec), the effect of freeze/thaw together with the short processing time resulted in low levels of reduction for *L. monocytogenes* (Fig. 13B). However, whether roast beef was fresh or frozen/thawed, the use of a combination short 60-sec radiant heat process followed by a 60- or 90-sec post-package pasteurization process (200°F) gave reduction levels exceeding 3 $\log_{10}$ cycles (Fig. 13B) that would have required a 10-min process to achieve by post-package pasteurization alone (Muriana et al., 2002). It should be noted that the combination process with fresh roast beef (60-sec radiant + 60-sec post-package) gave higher reductions than a slightly longer process (60-sec radiant + 90-sec post-package) using frozen/thawed roast beef (Fig. 13B). The reduced time spent in the post-package pasteurization phase of the combination process (60- or 90-sec) provided an additional benefit of generating little or no purge compared to what we observed in longer (4-, 6-, 8-, or 10-min) post-package pasteurization trials (Muriana et al., 2002). These data demonstrate the effectiveness of a short-

duration combined process to provide significant reduction levels and which provides additional processing after final packaging with no further handling of product. However, it should be clear that heat-treated product will need to be chilled using a brine chiller or blast cooler prior to boxing since the surface ¼-in has been heated.

We feel that radiant heat pre-package surface pasteurization, post-package surface pasteurization (Muriana et al., 2002), or a combination of the two processes, can alleviate potential *Listeria* contamination on RTE deli meat surfaces with minimal effect on product quality. The benefit should be considered in respect to the potential for acquisition of contamination that often exists in plant environments where RTE products are manufactured and packaged, and in comparison with pre-existing processing lines that do not include additional intervention steps. The potential savings of such a process must be measured in lieu of recent large recalls (and worse, illnesses and deaths) that have been attributed to the manufacture and distribution of contaminated RTE products. The data provided herein demonstrates that new processing strategies and microbial interventions are currently available that can provide safe products for the benefit of consumers and processors alike.

Acknowledgements

Support for this research was provided, in part, by the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK. The authors would also like to thank the following companies for technical discussion,

supply of product, and/or financial support of the research contained herein: Unitherm Foodsystems Inc., Stevison Hams, Vincent Giordano Corp., J. Freirich Co., Bar-S Foods, Sara Lee Corp., and Cargill Foods. We would also like to thank William Robertson, Brad Jordan, Suparna Mitra, and Bill Quimby for assistance with plating samples processed above.

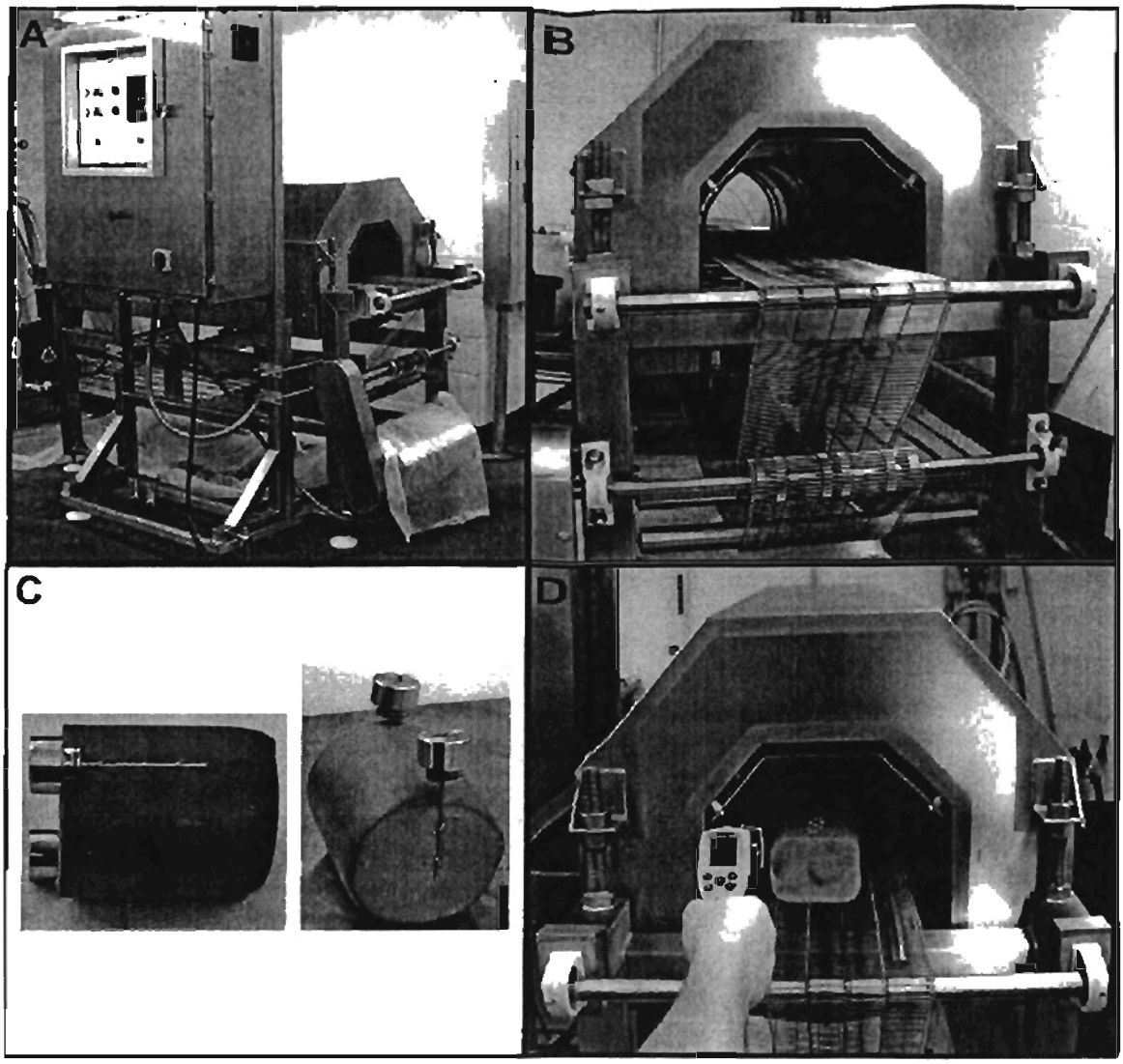

Figure 9. Radiant heat oven used in this study. Panel A, control box, conveyor belt, and radiant oven; Panel B, internal view of heating coils; Panel C, attachment of DataTrace™ hardened temperature probes to turkey bologna product; Panel D, Raytek ST80 hand-held infrared temperature monitor.

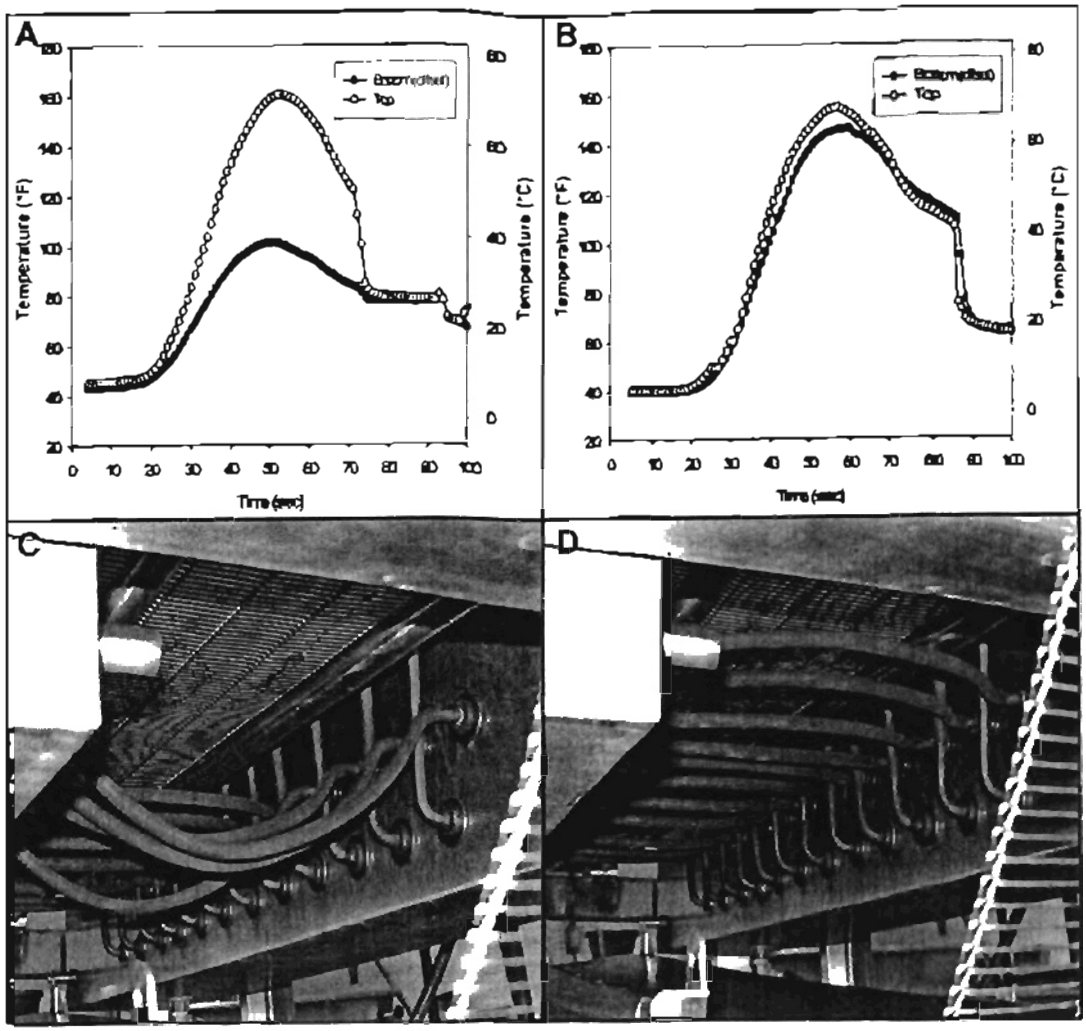

Figure 10. Temperature profiles obtained from turkey bologna using temperature-hardened DataTrace™ probes placed on top and bottom (offset from dead center) of product in relation to the positioning of the underlying heating elements. Panel A, temperature profiles when bottom heating elements were turned down and away from the conveyor belt, and Panel B, with bottom heating elements turned up toward the underside of the conveyor belt. Panel C, bottom heating elements directed downward, and Panel D, bottom heating elements positioned upward toward the conveyor belt. The radiant heat oven conditions were 60 sec residence time, temperature setting #4, and air temperature 475°F/246°C.

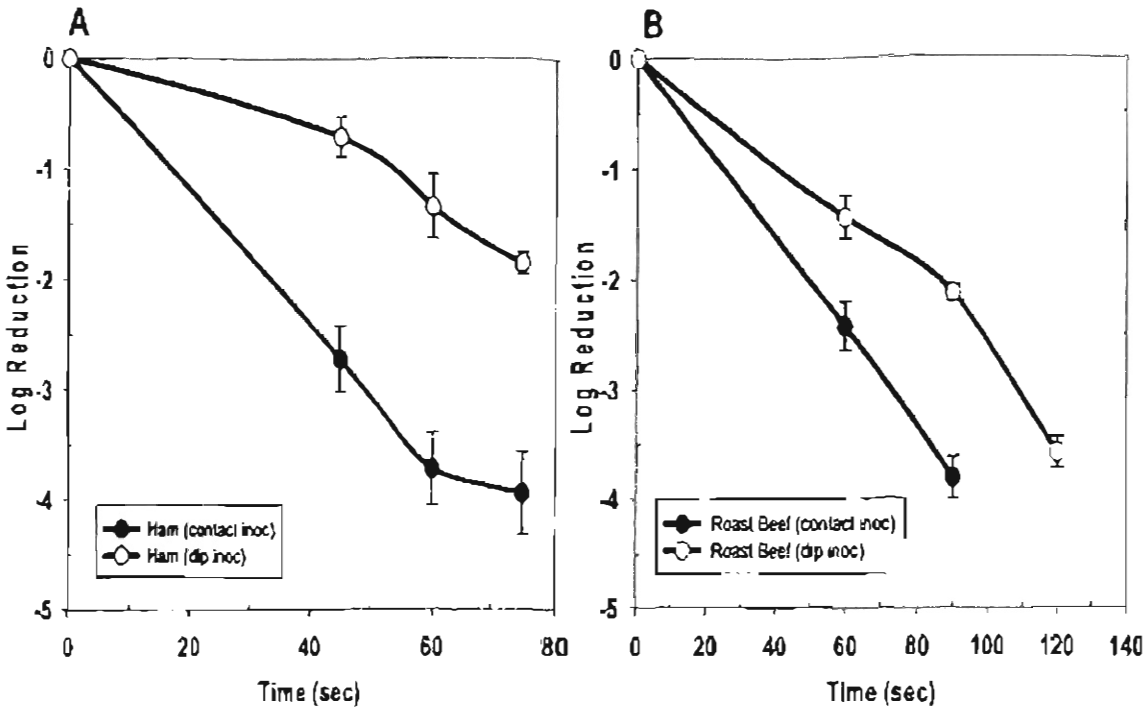

Figure 11. Radiant heat surface pasteurization of ham (Panel A) and roast beef (PanelB) inoculated by the dip or contact methods and processed at highest power setting for the time indicated. Each data point represents the mean of paired samples from triplicate replications. Error bars represent standard deviations of the mean.

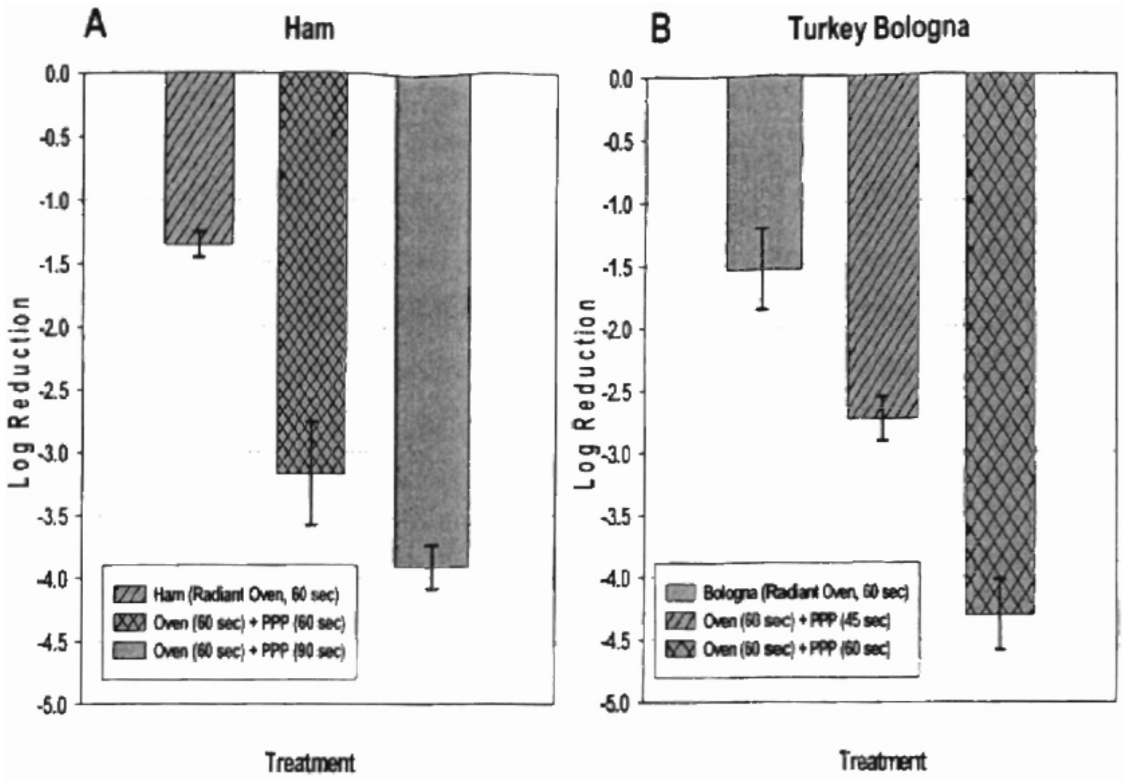

Figure 12. Radiant heat pre-package surface pasteurization of formed ham (Panel A) and turkey bologna (Panel B), and in combination with post-package pasteurization. Pre-package pasteurization (PPP) was performed at temperature setting #5 (highest) for ham (750°F/399°C air temperature) or #4 for bologna (475°F/246°C), with a 60 sec residence time for both products. Post-package pasteurization was applied for either 60 or 90 sec for ham, and 45 or 60 sec for turkey bologna at 200°F (93.3°C). Products were inoculated by the 'dip' method.

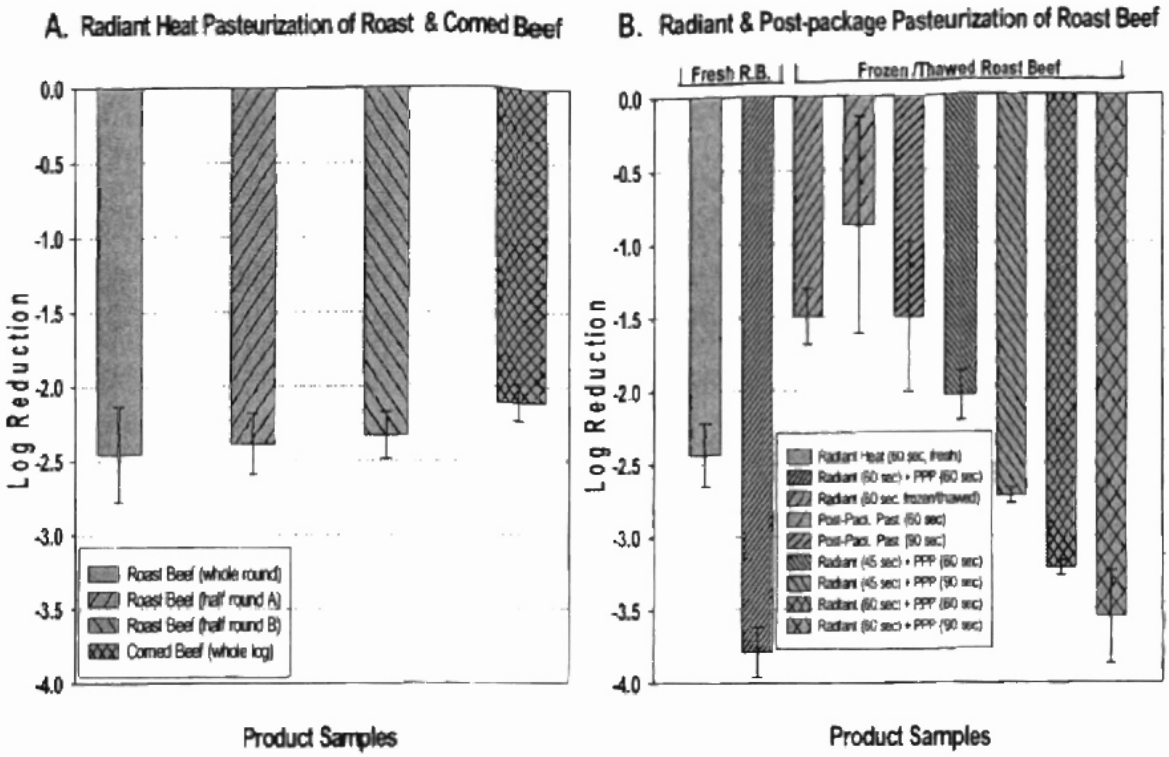

Figure 13. Radiant heat pre-package pasteurization of roast beef and in combination with post-package pasteurization. Panel A : radiant heat surface pasteurization of roast beef top rounds (whole round ; 13-16 lbs), half-rounds with cut-side placed facing tunnel exit ("A"; 6.5-8 lbs), half-rounds placed with cut-side facing to the side ("B"; 6.5-8 lbs), and corned beef logs (whole, 25-27 lbs). Panel B : radiant heat surface pasteurization alone, submersed water post-package pasteurization alone, and combination of pre- and post-package surface pasteurization of roast beef half-rounds. Treatments are as indicated on the figure. The entire Panel A and the first two bars of Panel B represent fresh, refrigerated product; the remainder of samples in Panel B represent product that was frozen and then thawed for testing. All samples were inoculated by the 'contact' inoculation method.

CHAPTER V

CONCLUSION

Post-processing contamination of RTE deli meats with *L. monocytogenes* has become a major concern to health authorities and food processing industries. Several current approaches described earlier have been suggested to reduce the levels of microbial contamination of meat and poultry products during processing. These require either relatively long treatment times with only minimal reduction in bacterial load or are ineffective for products with irregular surfaces. Our objective was to examine radiant surface heating (pre-package pasteurization), submersion water heating (post-package pasteurization) and a combination of pre-/post-package pasteurization as a means of reducing incidental surface contamination of *L. monocytogenes* on RTE meats (turkey bologna, deli roast beef, ham and deli turkey). Using radiant heat pre-package surface pasteurization, we were able to achieve a 1-3.5 $\log_{10}$ reduction with a 0.75-2 min process time at 450-750°F air temperature. Similarly for either a 2- or 3 min post-package pasteurization process we obtained 1.75-2.9 or 2.2-3.6 $\log_{10}$ reduction. However, a combination of pre- (1 min) and post-package (1 or 1.5 min) pasteurization provided 3-4 $\log_{10}$ reduction of *L. monocytogenes* with minimal effects on product quality and appearance. Contact-inoculation provided ~2 $\log_{10}$ greater reduction than the more extreme dip-inoculation method. These findings demonstrate that pre-package pasteurization either alone or in combination with post-package pasteurization provides an effective tool in controlling *L. monocytogenes* surface contamination that may have acquired

during post-processing handling and packaging. Pre-and post-package pasteurization are currently being used by large and medium sized meat processors and application of pre-and post-package pasteurization lethality steps can help establish a lower "risk category" for high risk products. The processes also help to reduce the FSIS sampling program in accordance with alternative 1 or alternative 2 of the recent "control of *L. monocytogenes* in RTE meat and poultry products; final rule" (USDA-FSIS, 2003)

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APPENDIX A

PRE-AND POST-PACKAGE PASTEURIZATION OF DELI TURKEY PRODUCTS FOR REDUCTION OF *LISTERIA MONOCYTOGENES*

PRE-AND POST-PACKAGE PASTEURIZATION OF DELI TURKEY PRODUCTS FOR REDUCTION OF *LISTERIA MONOCYTOGENES*

INTRODUCTION

L. monocytogenes is a significant foodborne pathogen and has been a public concern because of the numerous foodborne outbreaks and product recalls (Islam et al., 2002). *L. monocytogenes* is fairly ubiquitous on raw meats and in processing environments, requiring increased vigilance in worker and sanitation [procedures. The organism likely gains access to processed meats during packaging/re-packaging process through food contact surfaces, worker handling, and possibly air contamination (Tompkin et al., 2002). Deli turkey products have been involved with the largest deli turkey products in the U.S. history. Subsequently, another large listeriosis outbreak was epidemiologically linked to deli turkey products and was implicated in 29 cases of illness and 4 deaths, resulting in the recall of 17 million lbs of deli turkey products (CDC, 2000). Currently, a large foodborne outbreak of listeriosis of unknown food origin occurred in the Northeast, suspected of involving deli turkey, has resulted in 13 deaths and 43 illnesses. Isolates of *L. monocytogenes* from the deceased victims shared the same fingerprint pattern as that found among a non-foodcontact surface environment of one processor, resulting in a large recall of deli turkey products (28 million pounds) (USDA-FSIS, 2002). In response to the continuing detection and isolation of *L. monocytogenes* in RTE products and processing facilities, the USDA-FSIS has proposed more stringent testing

standards for facilities that make such products and has issued a zero-tolerance for *L. monocytogenes* in RTE foods based on the concept of adulterant and added agent (Shank et al., 1996).

Our objective was to examine pre-package, post-package, and combination pre-/post-package pasteurization of deli turkey products as a means of reducing possible *L. monocytogenes* surface contamination.

MATERIALS AND METHODS

Product samples:

Four different types of deli turkey products (oven roasted, peppered, naturally browned (skin-on) and oil browned) were provided by different processors for conducting the research. The product was stored at refrigeration temperature and removed just prior to the experiment.

Bacterial strains:

A four-strain mixture of *L. monocytogenes* (Scott A-2, V7-2, 39-2 and 383-2 similar to that used in the earlier study was used. The bacterial strains were inoculated by transferring 100 μl of thawed frozen culture into 10ml of Brain Heart Infusion (BHI) broth and incubated overnight at 30°C for use with contact inoculation the next day. These overnight cultures were mixed in equal proportions and the mixture was surface plated (100 μl) onto Tryptic Soy Agar (TSA) and held overnight at 30°C.

Product inoculation:

The products provided by the processors were removed from their packaging wrap and were inoculated by the contact inoculation method (Fig. 14). The process involves the use of sponge-foam padding material cut to the shape of a petri plate, sterilized and used to pick-up the mixed-strain inoculum lawn from inoculated petri plates after overnight incubation on agar plates using half-twist motion. The inoculum was then 'contact inoculated' onto the surface of the product using the same half-twist motion and the product was subjected to pre-package radiant heating.

Pre-package pasteurization:

Pre-package radiant heating was employed using a radiant heat oven (similar to earlier study). Inoculated products were passed through the radiant heat oven (Fig. 9) for either 60 or 75 sec at full (#5 dial setting) as desired by the processors. After passing through the oven, product samples were transferred into a sterile bag, chilled in an ice-water slurry and rinsed with 50 ml sterile diluent (BPW) to recover cells for microbial analysis. Inoculated but un-heated samples served as control. The procedure was repeated for all 4 types of turkey products.

Post-package pasteurization:

Post-package pasteurization was performed using a 50 gallon steam-injected temperature-controlled water bath (Muriana et al., 2002). The products were surface inoculated, vacuum packaged, and then post-package pasteurized for either 2.0, 3.0, 4.0 or 5.0 min as desired by the processors.

Combination pre-and post-package pasteurization:

A combination pasteurization process was examined by pre-package pasteurization treatment (1.0 min) of surface inoculated product followed by vacuum packaging and then post-package pasteurized by submersion heating for an additional 1.0 or 1.5 min at 200°F. The products were then cooled and then subjected to microbial analysis.

Product temperature measurement:

We used an infrared digital thermometer to measure the temperature on the surface of the product (similar to earlier study). Another temperature measuring device we tested that may have application in the future was a thermographic imaging camera whose digital diagnostics provide for a temperature measurement from every computer pixel in a designated boxed area (Fig. 18).

Microbial analysis:

After the treatment the remaining inoculum bacteria was recovered by placing the product into sterile bags into which 25-50 ml of sterile diluent (BPW) was added. The rinse buffer obtained after shaking the bags was pour plated using appropriate serial dilutions using TSA and incubated at 30°C for 48 hrs.

Experimental design:

All the experimental trails were performed in triplicate replications and in pairs at each processing condition within a replicate. Error bars were used to represent standard deviation of the means of triplicate replications.

Statistical analysis:

Data were analyzed by ANOVA using the general linear models (GLM) procedure of SAS Institute, Inc. (Steel and Torrie, 1960). Differences in mean log_{10} CFU/g among treatments were analyzed by a paired comparison t-test using SAS (version 8.2) at $p< 0.05$.

RESULTS AND DISCUSSIONS

Numerous outbreaks of foodborne illness and product recalls have been associated with RTE meat products due to *L. monocytogenes* post-processing contamination and there is an increasing need for post-processing hurdles to control *L. monocytogenes* in RTE meats (Islam et al., 2002). In addition to post-package pasteurization of deli hams and roast beef (examined earlier) we also processed 4 different types of deli turkey products for either 60 or 75 sec. Using pre-pasteurization method (Fig. 15) we obtained a 2.0-3.25 $\log_{10}$ reduction for 60 sec processing time and 2.8-3.75 $\log_{10}$ reduction with a 75 sec process. This level of reduction establishes radiant heat surface pasteurization as a post-lethality step to reduce risk of *L. monocytogenes*. Extended delay in packaging may result in environmental exposure and possible re-contamination of the product, thereby reducing the benefits obtained by pre-package pasteurization. Post-package pasteurization has the benefit that since the product is packaged, there is no chance for re-contamination. However, the presence of the packaging film acts as a barrier preventing quick surface heating as occurs with radiant heating. Using post-package pasteurization (Fig. 16) we obtained 1.95-2.81 $\log_{10}$ reduction, 2.01-3.02 $\log_{10}$ reduction, 2.51-2.94 $\log_{10}$ reduction and 2.82 $\log_{10}$ reduction for a processing time of 2.0, 3.0, 4.0 and 5.0 min respectively. The extent of reduction varied depending upon the type of product with oil browned giving the maximum reduction at all different processing times. Surface coloration may play a role in absorbing heat more/less than other types of products (i.e. oil browned product).

In order to overcome the limitations of each processes we examined pre-package pasteurization followed by post-package pasteurization while the product surface is still warm (by radiant heating). When pre-package pasteurization (1 min) of oven-roasted turkey was followed by either a 1.0 min or 1.5 min post-package pasteurization step at 200°F (93.3°C), we obtained a combined 3.14 $\log_{10}$ reduction or 3.76 $\log_{10}$ reduction respectively (Fig. 17). Thus combined process not only reduces the amount of processing time required for post-package pasteurization but also reduces the amount of purge. The potential savings of such a process must be measured in lieu of recent recalls, diseases and outbreaks attributed to the manufacturer and distributors for the distribution of contaminated products. This provides a new processing strategy and microbial interventions that can provide safe products to both consumers and processors.

Thermographic imaging of a deli turkey breast product (Fig. 18A-B) in 'minimum temperature' mode (within the boxed area) indicated that the lowest temperature in the designated surface area was 25.6°F (-3.6°C). Similarly, during processing, we demonstrated minimum temperatures (within the box) at several positions within, and upon exit, of the radiant heat oven (Fig. 18C-D). When a process such as this is dependent upon surface pasteurization that is not intended to 're-cook' the product, then any surface areas that have not reached thermicidal levels could be weak links in the processing event. The use of such an imaging system could provide important feedback to modulate the residence time itself if combined with computer control of belt speed or a reprocessing (i.e.,

rejection) mechanism. It is conceivable that the computerized control of temperature sensing by this method could be used for continuous monitoring of processed product whereby those sample pieces that fall below a specific surface minimum temperature (that have already been correlated to internal temperatures), would be removed from the line for possible rework or reprocessing.

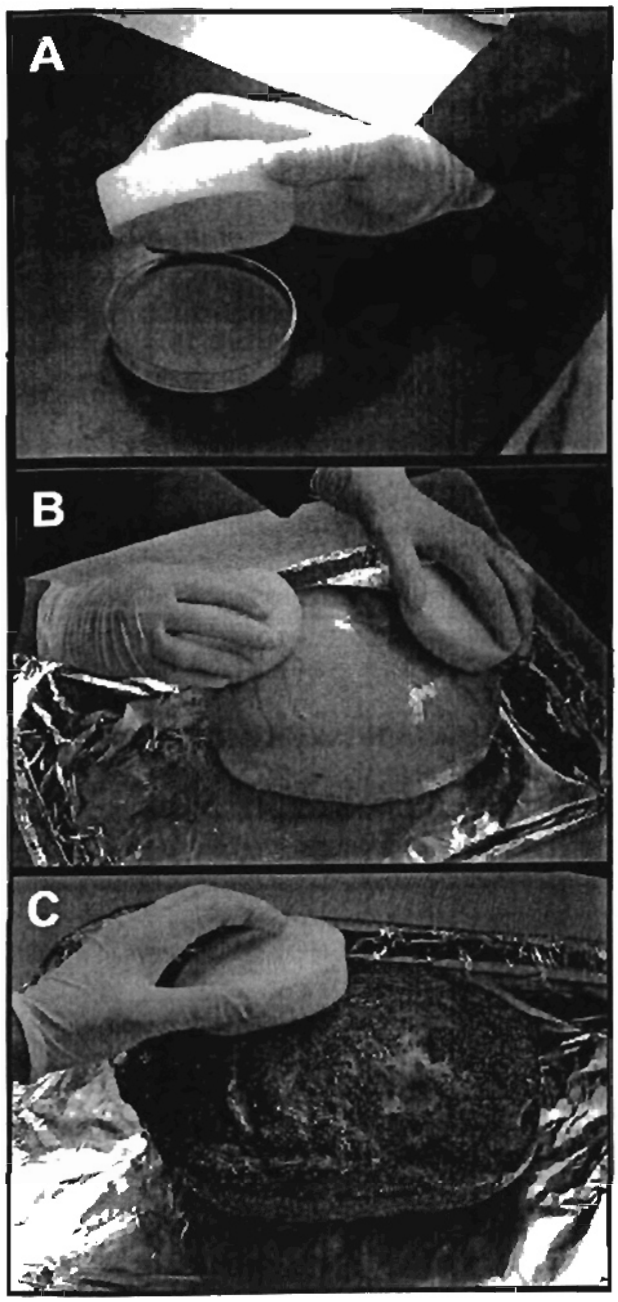

Figure 14. Surface 'contact inoculation' using sterile round sponge padding material. Panel A, taking up inoculum from petri plates. Panels B and C, inoculum is applied to RTE deli meat products by a twist motion upon contact.

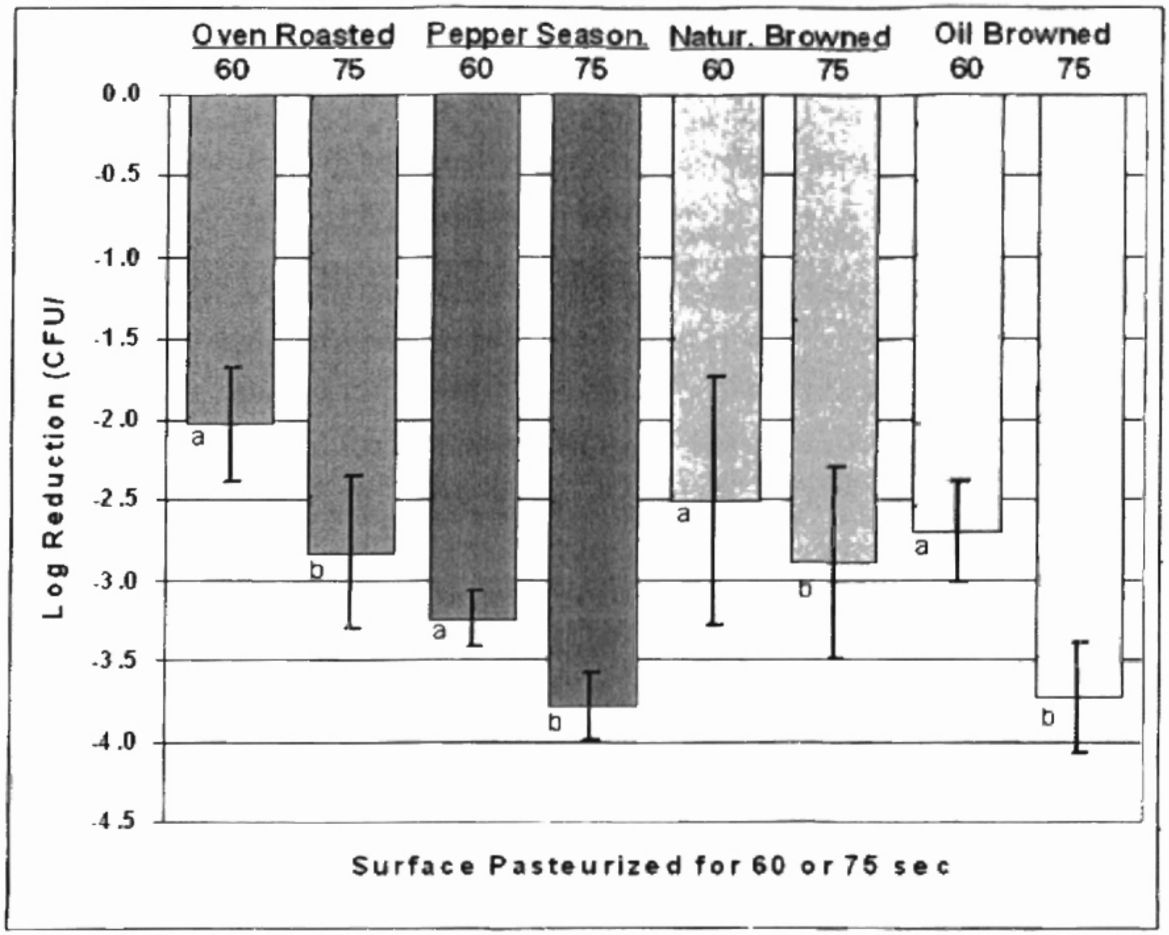

Figure 15. Pre-package surface pasteurization of four types of deli turkey (oven roasted, pepper seasoned, naturally-browned/skin-on, and oil browned) using a radiant heat oven. Oven dwell time was either 60 or 75 seconds as indicated. Error bars represent +/- standard deviation of the means of triplicate replication. Significant differences of treatment times for a given product were determined using a paired t-test. Bars within a specific product with different letters (a, b) are significantly different ($p < 0.05$)

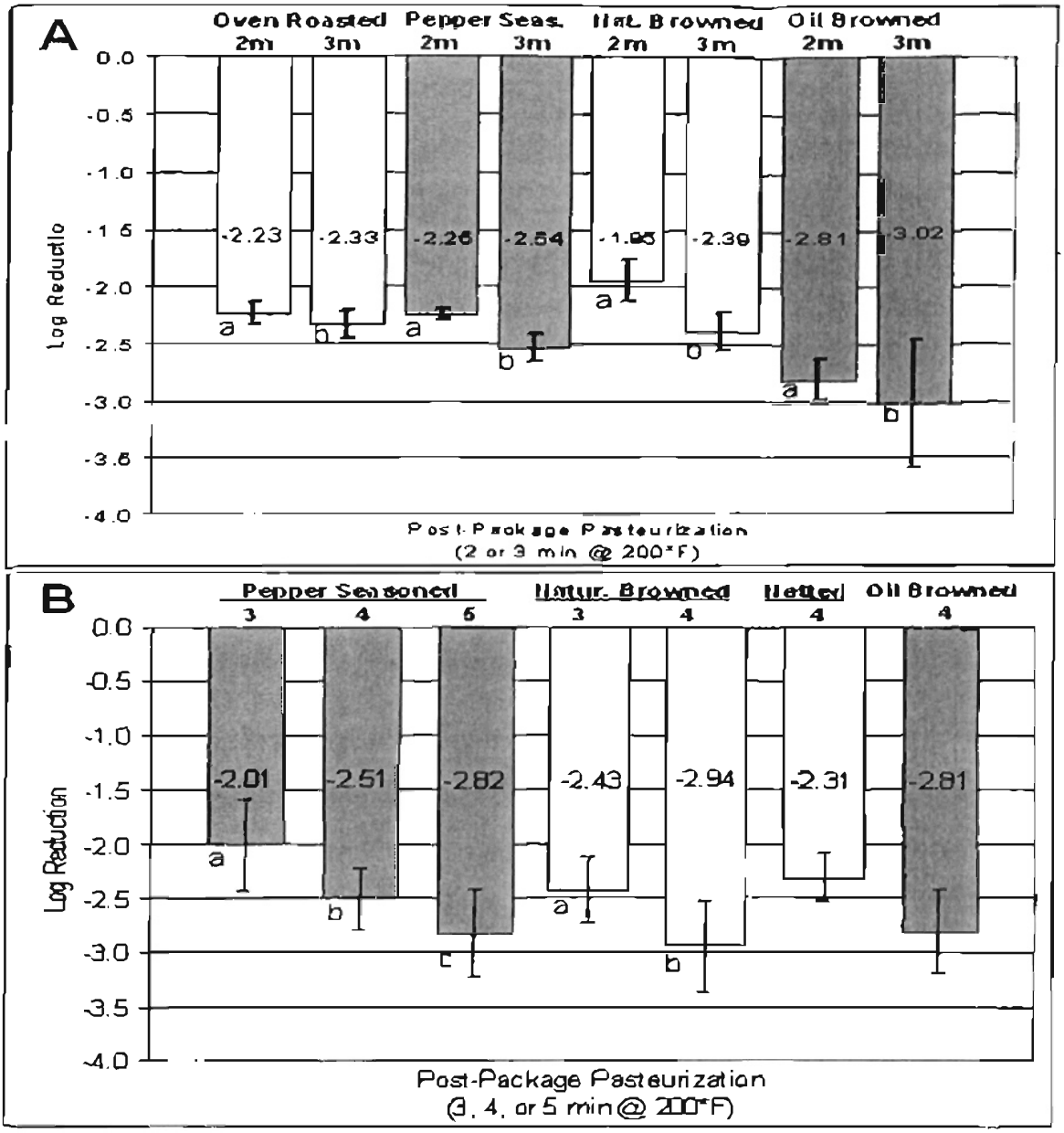

Figure 16. Post-package surface pasteurization of four types of deli turkey (oven roasted, pepper seasoned, naturally-browned/skin-on, and oil browned) using submersion heating in steam-injected water (200°F). Panel A, post-package pasteurization for 2 and 3 minutes (product from manufacturer A); Panel B, post-package pasteurization for 3, 4, or 5 min (product from manufacturer B). Error bars represent +/- standard deviation of the means of triplicate replications. Significant differences of treatment times for a given product were determined using a paired t-test. Bars within a specific product with different letters (a, b) are significantly different ($p < 0.05$)

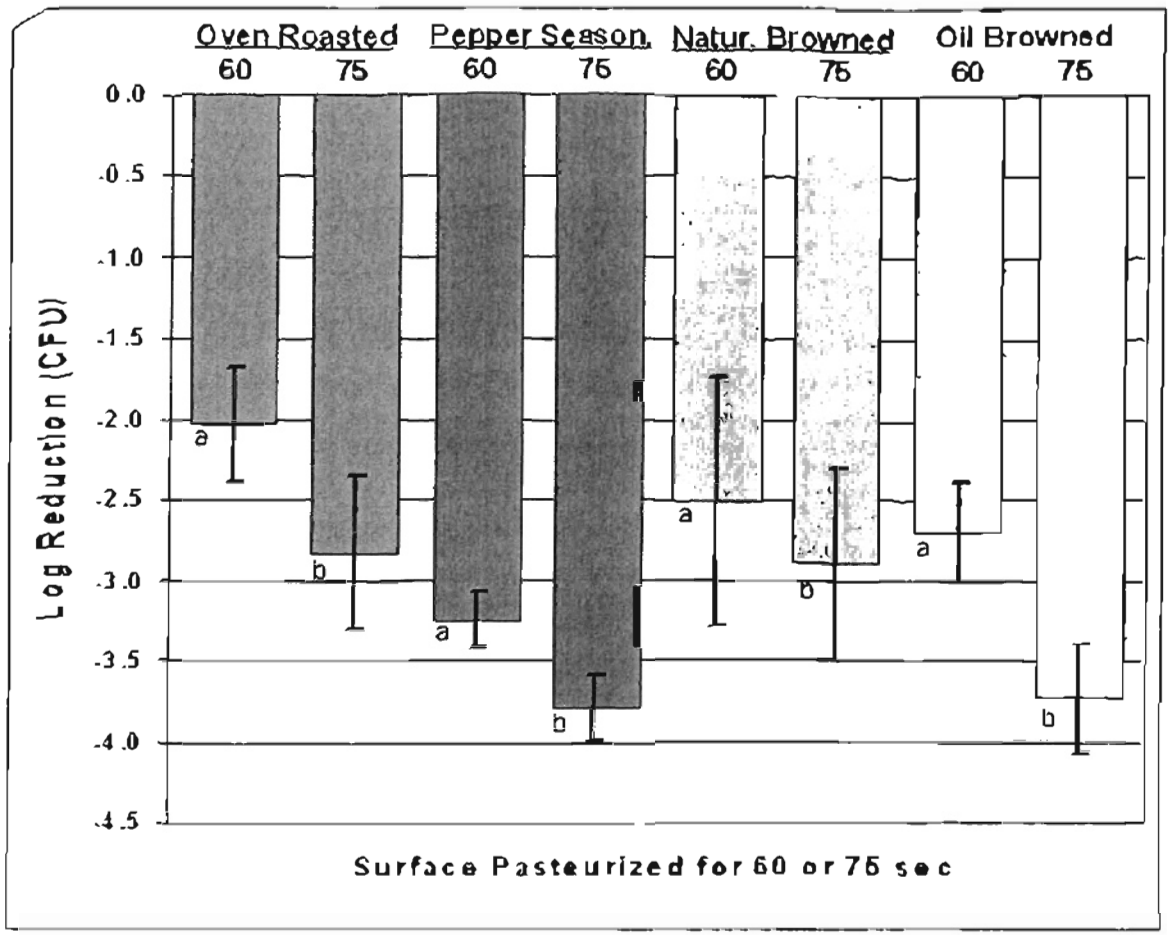

Figure 17. Combination pre- and post-package pasteurization of oven-roasted turkey. Contact-inoculated deli turkey was pre-package pasteurized for 1 min, vacuum-packaged, and post-package pasteurized for an additional 1.0 or 1.5 min at 200°F. Significant differences of treatment times for the same process were determined using a paired t-test. Bars within a specific product with different letters (a, b) are significantly different ($p < 0.05$)

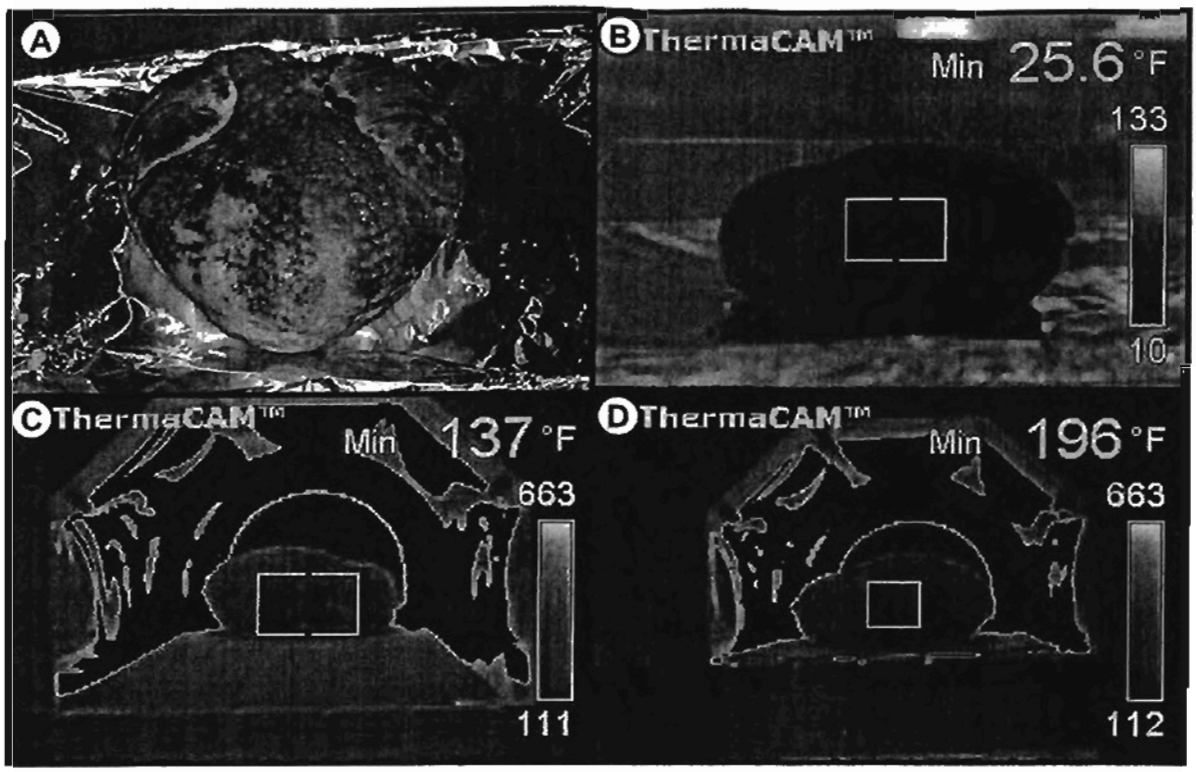

Figure 18. Thermographic imaging of a deli turkey breast. Naturally-browned (skin-on) deli turkey (panel a) examined by thermal imaging before (panel b), during (panel c), and after (panel d) pre-package pasteurization through a radiant heat oven.

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APPENDIX B

EXTENDED SHELF-LIFE STUDY OF ROAST BEEF (half-rounds) AFTER SURFACE PASTEURIZATION USING INFRARED GRILL

EXTENDED SHELF-LIFE STUDY OF ROAST BEEF (half-rounds) AFTER SURFACE PASTEURIZATION USING INFRARED GRILL

INTRODUCTION

Food contamination creates enormous social and economic burdens on countries and their health systems. A survey conducted by Center for Science in the Public Interest (CSPI) revealed that contaminated food causes upto 76million illnesses, 325,000 hospitalizations and 5000 deaths every year in U.S. alone. As a result, governmental institutions and food industries must control the contamination of raw and finished products (Dewaal, 2003). Recontamination of precooked meat products is a potential source of foodborne illnesses.

The research conducted in the previous chapters involves the use of pathogens to validate the application of pre- and post-package pasteurization to reduce *L. monocytogenes* in RTE meats. The purpose of this study is to examine the efficacy of the pre-package pasteurization (using IR grill oven) in extending the shelf-life of RTE roast beef and facilitate our earlier studies.

MATERIALS AND METHODS

Product samples:

The beef samples were provided by a commercial beef processor and were stored at 3°C upon receipt and removed from refrigerated storage just prior to treatment. The treated products were stored at 3°C for 0, 15, 30, 45, 60 and 75, 90 days and were maintained at this temperature until analysis.

Pre-package pasteurization:

The radiant heat oven used in the earlier study was used to provide surface pasteurization of RTE roast beef. The product was passed through the radiant heat grill for 1min at full heat (#5 dial setting). After passage, the samples were transferred into a sterile bag, vacuum packaged and shrinked for 5sec (using hot waterbath at 195°C). The packed product was then chilled before storage at refrigeration temperature, to facilitate shelf-life study. The product temperature was measured using an infrared digital thermometer. Products that served as controls were opened repackaged and shrink wrapped, cooled until microbial analysis.

Microbial analysis:

The "0" day samples (both heated and unheated control) were analyzed by rinsing with 50ml of sterile diluent (0.1% BPW) and the rinse was recovered for total plate count. This was followed by appropriate serial dilutions and was pour plated using Plate Count Agar (PCA) followed by incubation at 30°C for 48hrs.

Experimental design:

Trials were performed in triplicate and samples were run in pairs for both control and surface pasteurized treatment within a replicate. Standard deviation was obtained for the triplicate replications for means of sample pairs.

Statistical analysis:

Data were analyzed by ANOVA using the general linear models (GLM) procedure of SAS Institute, Inc. (Steel and Torrie, 1960). When a significant F-statistics was noted, treatment means were separated by the Tukey's multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSIONS

In general, nitrites are used to extend shelf-life and provide microbial safety. Concerns about nitrosamine formation have lead to concentration on alternate techniques to maintain shelf-life of meats (Maca et al., 1999). Surface pasteurization of meats is becoming an effective means of reducing post-processing contamination of RTE meats. Such a treatment not only reduces the risk of contamination with pathogens but also aids in longer storage period.

In this study we examined the shelf-life of pre-package pasteurized roast beef stored at refrigeration temperature for 0, 15, 30, 45, 60, 75 and 90 days. The results (Fig.19) indicate that using pre-package pasteurization we were able to extend the shelf-life of the product for 90 days. The total plate count for the treated samples remained acceptable until 90 days, and was only slightly higher than the "0" day count. As the storage time increased from 60 to 90 days the total plate count for the treated samples started decreasing, which can be due to excess inhibitory substances (acids) produced by the residing microbes. The total plate count for the control increased with increased storage time and was atleast 2.5 $\log_{10}$ cycles higher than that of treated samples. This trend was observed from day 15 to day 60. The total plate count for the control decreased from day 60 to day 75 and then again increased from day 75 to day 90 (can be due to growth of other organisms growing at the storage temperature). Therefore this study validates the application of radiant heat pasteurization to extend the shelf-life of refrigerated meats. We propose this method as an effective process

to reduce incidental contamination acquired during post-processing handling, thus providing processor and consumer satisfaction.

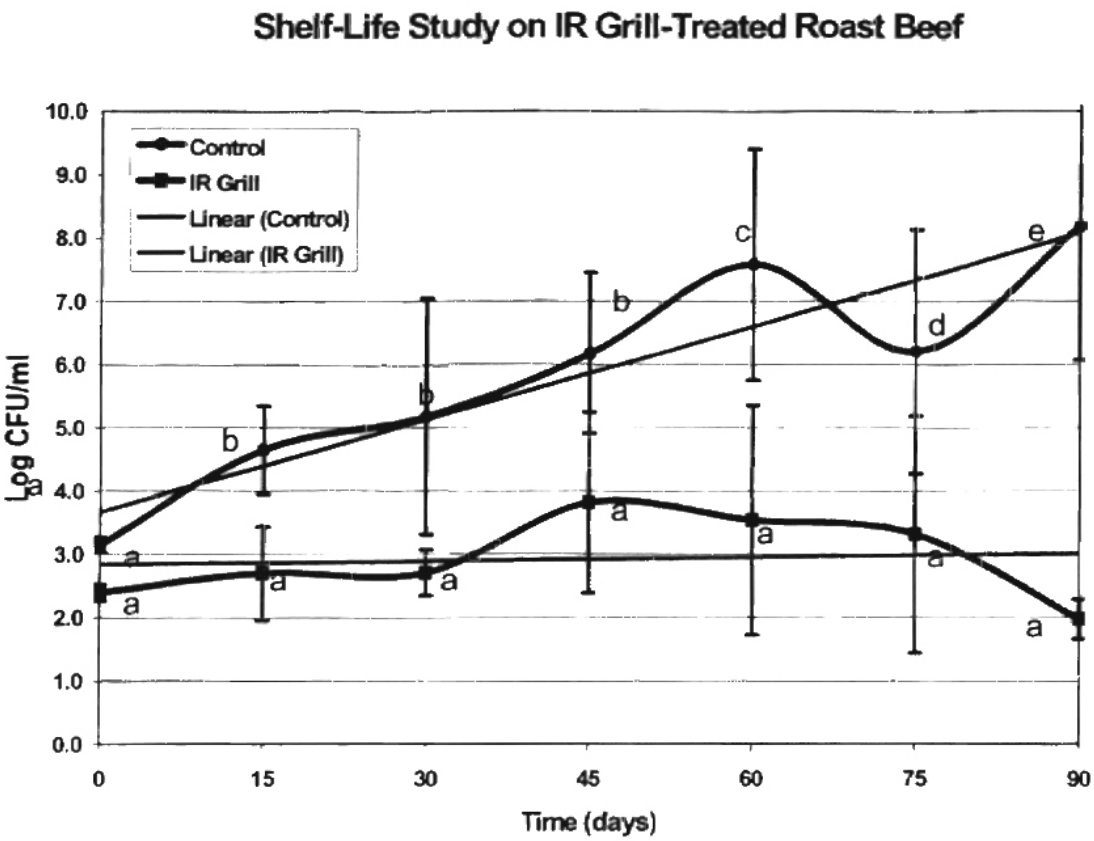

Figure 19. Shelf-Life Study of IR Grill-Treated Roast Beef. Non-heated control (upper curve) was sampled for total plate count at 0, 15, 30, 45, 60, 75, and 90 days. Radiant heat surface pasteurized roast beef (lower curve) was sampled after the same shelf-life period. Error bars represent +/- standard deviation of the means of triplicate replications. Significant differences between consecutive sampling data within a treatment were determined using ANOVA (GLM, Tukey's method of multiple comparisons). Data with the same letter designation are not significantly different ($p<0.05$)

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