Efficacy of Liquid Smoke as an Ingredient in Hotdogs against *Listeria monocytogenes* and its Effects on the Microbial Shelf-life and Quality Attributes

by

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Abstract

Research was conducted to study the efficacy of a commercial liquid smoke as an ingredient in hotdog manufacture against *L. monocytogenes* as well as its effect on the microbial shelflife and quality attributes during storage. Chicken and pork hotdogs were made with 0, 2.5, 5 and 10% w/w liquid smoke as an ingredient along with spices. These hotdogs were inoculated with high (8 $\log_{10}$ CFU/ mL) or low (4 $\log_{10}$ CFU/ mL) levels of *L. monocytogenes* serotype 4b, vacuum packaged and stored at 4°C for up to 12 weeks. Sampling was conducted every week for 12 weeks to estimate growth of *L. monocytogenes* growth, spoilage microflora (aerobic plate counts, yeast and molds, lactic acid bacteria and total coliforms), sensory attributes and texture profile analysis.

Liquid smoke was effective ($p \leq 0.05$) in reducing the growth of *L. monocytogenes* throughout the storage period as compared to the control (0% Liquid smoke) samples. Hotdogs made with 10% liquid smoke completely suppressed the growth of *L. monocytogenes* at high and low inoculum levels. On the other hand, growth of spoilage organisms below detection limit (7.5 CFU/mL) on all the treatments throughout the storage period indicated that the product was handled hygienically from manufacture to packaging.

Addition of liquid smoke did not affect ($p > 0.05$) the sensory properties of the hotdogs. Untrained panelists rated the products “like moderately” to “like slightly” for appearance, texture, juiciness, flavor and overall acceptability. They did not perceive

differences ($p>0.05$) among the hotdog treatments as compared to the control samples throughout the storage period.

Texture profile analysis indicated that incorporation of liquid smoke affected ($p\leq0.05$) the texture and chewiness of the hotdogs. Although addition of 10% liquid smoke had a significantly greater hardness and chewiness than the control samples, taste test indicated that taste panelists were not able to detect the differences.

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List of Abbreviations

RTE	Ready-to-eat
L. monocytogenes	*Listeria monocytogenes*
CFU/mL	Colony Forming Units per milliliter
UV	Ultraviolet

CHAPTER I

GENERAL INTRODUCTION

Listeria monocytogenes is a major foodborne pathogen responsible for high hospitalization rates and the highest fatalities as compared to other pathogens related to foods. Consumption of *L. monocytogenes* contaminated foods can result in life threatening conditions in elderly individuals, pregnant women, newborns, immunosuppressed and immunocompromised individuals. However, there are instances where apparently normal healthy individuals have exhibited listeriosis in both food borne epidemics and sporadic illnesses. Hence, the United States Department of Agriculture (USDA) has a "zero tolerance" policy towards *L. monocytogenes* in ready-to-eat (RTE) foods. In addition to foodborne illnesses, *Listeria* related food recalls have caused severe economic losses to the food industry. Although the industry applies strenuous efforts to eliminate *L. monocytogenes* in RTE foods, possible post-processing *Listeria* contamination has led to frequent recalls of RTE products as seen from the FSIS Recall Case Archive.

High prevalence of *L. monocytogenes* on foods is due to its ubiquitous nature and its ability to survive various processing stresses making it a difficult pathogen to eliminate. *L. monocytogenes* has been isolated from water, soil, vegetation, sewage, animal feeds, farms, foods and food-processing environments. Moreover, it can survive and grow at a wide temperature range of – 0.4 to 50°C, pH of 4.4 to 9.4 and water activity of $\geq$ 0.92. Researchers have also reported that *L. monocytogenes* consists of a number of serovars exhibiting varying pathogenicity and sensitivities to food processing

conditions. Although all the serovars are sensitive to high processing temperatures, they can grow under refrigeration causing a food safety concern in RTE foods.

Listeria monocytogenes from the environment enters the food processing facility through the workers and raw materials. In the case of poultry, it can settle on the skin, feathers and legs of chicken at the farm and get carried to the processing plants. Once inside the processing plant, *L. monocytogenes* can contaminate the processing equipment such as conveyor belts, and tables and disseminate on other products. *L. monocytogenes* contaminated chicken parts form an important source for introducing the pathogen in RTE plants. The pathogen rapidly adjusts to the low temperature and establishes itself in nutrient rich wet food processing.

Once established, *L. monocytogenes* co-exists and interacts with other bacteria in the processing environment which can have either detrimental or beneficial effect on the survival and growth of the pathogen. *L. monocytogenes* can interact with its own species or different species forming biofilms covered with extracellular polysaccharides. Such single or multi-species biofilms protects the pathogen from desiccation, sanitizers and radiation. The biofilms act as a source of dissemination of *L. monocytogenes* on RTE foods.

Consumption of foods such as coleslaw, raw vegetables, milk and Mexican-style cheese have been linked to initial listeriosis outbreaks but recent outbreaks have been associated with RTE meat and poultry products such as hotdogs and deli loaves. Consumption of *L. monocytogenes* contaminated foods has led to 31 outbreaks from 1998-2011 with 578 associated illnesses. These outbreaks have not only affected human

health but had a significant impact on the food industry causing some processors to suspend their operations.

In order to protect the health of the consumers and produce *Listeria*-safe foods, the industry follows the *L. monocytogenes* control program. The program involves guidelines for plant design and construction, compartmentalization of different processing areas, drains, air flow and movement of workers, raw material and final products. It also considers the importance of equipment design and their sanitation procedures to prevent the attachment and growth of *L. monocytogenes*. Success of this program is validated by sampling food-contact and non-contact surfaces for presence of *L. monoocytogenes*.

In addition, the USDA-FSIS has formulated three alternatives for RTE food manufacturers as a part of *Listeria* control program. The USDA-FSIS states that the food processors can either use an antimicrobial intervention and/or post-process intervention step to eliminate *Listeria* from the product and prevent growth during storage (alternative 1 and 2). In addition, food processors can completely rely on their sanitation program (alternative 3) with frequent USDA-FSIS verification testing.

Food manufacturers use synthetic or natural antimicrobial interventions to reduce and/or eliminate *L. monocytogenes* from RTE products. Synthetic antimicrobials such as the salts of lactic acids are commonly used in RTE meat and poultry products. These chemicals have been used in RTE products to eliminate *L. monocytogenes* and suppress their growth during storage. Although, these chemicals are highly effective, they can affect the organolpetic properties of foods. Moreover, consumers are demanding “all natural” foods with less or no synthetic antimicrobials added. To meet consumer demand,

the food industry is trying to maintain a “clean label” by using fewer additives in the products and still maintaining food safety.

Natural extracts from various herbs, spices and plants exhibiting antimicrobial properties can be potential alternatives to synthetic antimicrobials. Plant extractives contain bioactive compounds that can affect the ion transport, interfere with ATP generations and can inhibit enzymatic activities. Researchers have reported that oils from various herbs and spices have antimicrobial chemicals which act against foodborne pathogens such as *L. monocytogenes*, *Escherichia coli*, and *Salmonella* spp.

In addition to plant extractives, wood smoke has a potential to be used as an antimicrobial in RTE foods. Smoke contains a variety of carbonyls, phenols, aldehydes, ketones and hydrocarbons which not only impart color, flavor and texture to the product but also exhibits antimicrobial properties. Smoking of meat is an age-old preservation technique. Researchers have been successful in identifying the antimicrobial chemicals from smoke and incorporating them in a liquid form for easy application. Liquid smoke is generally used as a spray or dip for RTE products such as hotdogs to reduce and/or eliminate *L. monocytogenes* ; research on its use as an ingredient is lacking.

We hypothesize that the application of liquid smoke as an ingredient in hotdog formulation can reduce and/or eliminate *L. monocytogenes* without affecting shelf-life and organoleptic properties of the product during storage. Such application will eliminate with use of synthetic antimicrobials like lactate and diacetates as well as can replace functional additives for improving texture thus following the market trends of “clean label” and “all natural products”.

CHAPTER II

LITERATURE REVIEW

Listeria monocytogenes has emerged as one of the most important and lethal food borne pathogen resulting in a high rate of hospitalization (94%) and the highest fatality rate (19%) amongst all food borne illnesses (Scallan et al. 2011). Although initial outbreaks of listeriosis were linked to consumption of coleslaw, raw vegetables, milk and Mexican-style cheese, the consumption of undercooked chicken frankfurters has been strongly linked epidemiologically to an increased risk of listeriosis (Schwartz et al. 1988).

The ubiquitous nature of *Listeria* and its wide distribution in the environment along with its ability to survive in a wide range of temperatures (0 to 45°C), pH (4.4 to 9.4), and water activity of $\geq$ 0.92, makes it a pathogen of concern in food processing (FAO/WHO, 2004). *Listeria* enters the processing environment at low intensities and subsequently establishes itself in wet places such as conveyor belts, floors, drains, and other transporting equipment (Gudbjörnsdóttir et al. 2004). These niches therefore act as a source for the dissemination of *L. monocytogenes* on cooked food (CDC, 2002).

Consumption of *L. monocytogenes* contaminated foods can result in life threatening conditions in elderly individuals, pregnant women, newborns, immunosuppressed and immunocompromised individuals (CDC, 2002). However, there are instances where apparently normal healthy individuals have exhibited listeriosis in both food borne epidemic and sporadic illnesses (Farber and Peterkin, 1991). Hence, the United States Department of Agriculture (USDA) has a “zero tolerance” policy towards *L. monocytogenes* in ready-to-eat (RTE) foods. Although the industry applies strenuous efforts to eliminate *L. monocytogenes* in RTE foods, possible post-processing *Listeria*

contamination has led to frequent recalls of RTE products as seen from the FSIS Recall Case Archive (http://www.fsis.usda.gov/Fsis_Recalls/Recall_Case_Archive/index.asp).

The importance of *L. monocytogenes* in foods warrants a need for better understanding of the pathogen. Hence this review focuses on the taxonomy and diversity in genus *Listeria*, the biochemical structure of the cell and intervention strategies used to eliminate and/ or reduce *L. monocytogenes* in foods.

TAXONOMY OF *LISTERIA* SPP.

Murray and co-researchers (1926) were the first to describe *Listeria* as *Bacterium monocytogenes* while Pirie (1927) classified similar gram positive bacillus causing "the Tiger River disease" as *Listerella hepatolytica* and reclassified it as *Listeria* in 1940 (Pirie, 1940). Genus *Listeria* is comprised of *grayi*, *murrayi*, *innocua*, *ivanovii*, *welshimeri*, *seeligeri*, *marthii* and *rocourtiae* (Rocourt and Buchrieser, 2007; Graves et al. 2010; Leclercq et al. 2010). These species have been further classified by serotyping based on surface somatic and flagellar antigens.

Listeria monocytogenes, a major foodborne pathogen, is composed of 13 serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4ab, 4c, 4d, 4e, and 7) grouped into three phylogenetic lineages providing genetic information on serotype evolution. Lineage I (LI) comprise of serotypes 1/2b, 3b, 4b, 4e, 4d, 4ab and 7; lineage II (LII) contains serotypes 1/2a, 1/2c, 3a and 3c while lineage III (LIII) consist 4a, 4b and 4c. Most researchers assigned 4b to lineage I but Ward et al. (2004) for the first time assigned it to both LI and LIII indicating differences in strains and/or methodology used to form lineages. L I, II and III comprise of *Listeria monocytogenes* serotypes mainly associated

with human listeriosis, food and animals respectively (Graves et al. 1994; Ward et al. 2004; Ragon et al. 2008).

Lineages evolved due to the mosaic core genome of *Listeria* where some core genes had undergone homologous recombination across lineages or species and some core genes evolved under the selective niche specific pressure (Dunn et al. 2009). During evolution, LI was probably not exposed to significant genetic transfers as reflected in its recent coalescence of haplotypes (Ward et al. 2004). Within LI, serotype 3b, 4b and 7 evolved from serotype 1/2b. Serotype 4b appeared only once during LI evolution and underwent genetic changes to increase virulence and further evolved into highly pathogenic clones. Serotypes 4d and 4e were derived from 4b while 4a is an evolutionary intermediate between 1/2b and 4b and 1/2a is ancestor of 1/2c (Ragon et al. 2008; Chen et al. 2009). Such evolutionary information can help in determining groups with common ancestors and taxonomic reclassification of *Listeria monocytogenes*.

Weidmann et al. (1997) suggested that LIII should be recognized as a subspecies since LIII has a small number of isolates, distinct ribotype fragment, 16s rRNA sequence differing in at least 2 nucleotides and DNA-DNA homology. Ward et al. (2004) argued that lineage I and III belong to same clade, recognizing LIII as a species/subspecies would be against modern systematic principle and suggested that both the lineages be reclassified into two new species. Ragon et al. (2008) supported the claim based on their finding that lineages did not have a single common allele among them warranting distinction of those lineages into three separate species.

In addition to the delineation of three lineages into separate species, heterogeneity among the same serotypes can form the basis for reclassification. Ericsson et al. (1995)

subtyped serovar 4b into 2 PCR-restriction analysis (PCR-REA) groups while Unnerstad et al. (1999) divided 1/2a isolates from humans, animals, food and environment into two groups (irrespective of their origin) by PCR-REA. In another study, Giovannacci et al. (1999) grouped 241 strains isolated from pork slaughtering and cutting plant of serotype 1/2a in 16 genotypes while 6 strains of 3a grouped in 2 genotypes. Similarly, Cabrita et al. (2004) observed heterogeneity in the virulence potential among strains of serotypes 1/2a, 1/2b and 3b isolated from different foods.

Many researchers have tried to study the diversity by using techniques like the pulse-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis, random amplification of polymorphic DNA etc. These researchers also use different strains available to them or related to their study area. Data generated from such studies do point towards the intra-serotypic differences but non-uniformity in methodology and strains can be an obstacle in making a firm statement about presence of subspecies/subtype. It can be also argued if the differences found among the strains of same serotype are enough to distinguish it as a subspecies/subtype.

Listeria is a very diverse genera and its taxonomy is still in its infant phase. The description of whole genome of *Listeria* will also provide new insight in its taxonomic position.

BIOCHEMICAL CHARACTERISTICS OF *LISTERIA MONOCYTOGENES*

Listeria monocytogenes is a gram positive, catalase positive, oxidase negative bacteria, that has a unique ability to survive food processing conditions. *Listeria* can grow from pH 4.5 to 9.2; up to 10% (w/v) NaCl, 1-45°C under aerobic, microaerophilic and anaerobic conditions (FAO/WHO, 2004). It can occupy different food production

niches, survive, grow and contaminate food products. Tremendous efforts are being made to remove *Listeria* from foods and food processing environments. In-spite of all the efforts, *Listeria* still is a concern amongst food processors and food inspection authorities. Even before applying various intervention technologies against *Listeria*, it is important to understand its biochemical make-up which makes it tolerant to an array of parameters.

Listeria monocytogenes has a thick cell wall consisting of a peptidoglycan layer made from alternating units of N-acetylmuramic acid and N-acetylglucosamine linked via β-1, 4 linkages. The amino-sugars are cross linked by L-alanyl-γ-D-glutamyl-meso-diaminopimelyl-D-alanine-D-alanine. Covalently bound to peptidoglycans via a phosphodiester linkage, *L. monocytogenes* cell wall contains acidic polymers of ribitol-phosphate substituted with D-alanine and diverse sugars called teichoic acid (TA) imparting an overall negative charge to the cell. Additionally, amphiphilic lipoteichoic acids (LTA) are non-covalently anchored to the plasma membrane. The LTA of *L. monocytogenes* consists of a hydrophilic region of poly(glycerophosphate) partly substituted by ester linked D-alanine or D-galactose residues. The hydrophilic region is covalently linked to lipophilic glycol- or phophatidylglycolipids or phosphatidyl moieties consisting C15:0 anteiso and C17:0 anteiso as major fatty acids (Hether and Jackson, 1983; Uchikawa et al. 1986; Navarre and Schneewind, 1999).

These TA and LTA vary in composition (either qualitative or quantitative) within different species of genus *Listeria* and serotypes of *L. monocytogenes*. The differences in the composition of TA lead to alteration in its conformation and serological properties used to distinguish *L. monocytogenes* into various serotypes. The TA of *L.*

monocytogenes 4d contain high amount of glucose (1480 nmol/mg TA) which is absent in 4a and negligible in 3a (283 nmol/mg TA). Furthermore, TA of 4a has about 30 monomeric units of GlcNAcβ(1→2) ribitol phosphate linked via phosphodiester bond between C1 of ribitol and C4 of D-glucosamine. Compared to 4a, 4d has a more complex structure composed of 33 monomeric units of Glcβ(1→3)GlcNac(1→2)ribitol-phosphate linked by phosphodiester bonds between C5 of ribitol and C4 of D-glucosamine residues (Fujii et al. 1985). Teichoic acid structures of serotype 3a contain monomers of GlcNAc(α1→2/4)ribitol-phosphate; 4b Gal(α1→6)[Glc(β1→3)]GlcNAc (α1-2/4)ribitol; 4f (fragment FI)Gal(α1→6)[GlcNAc(α1→3)]GlcNAc(β1→2/4)ribitol and Gal(α1→6) GlcNAc(β1→2/4)ribitol for fragment FII; and 6 GlcNAc(β1→2/4)ribitol (Uchikawa et al. 1986). These structural differences are possibly due to the evolutionary pattern of *L. monocytogenes*.

On the other hand, LTA of genus *Listeria* is composed of glycerol, galactose and glucose but *L. grayi* and *L. murrayi* lacks galactose moiety indicating a distant phylogenetic relation with other *Listeria* spp. Although serotypes 4a and 4b have approximately similar concentrations of these three compounds, serotype 4a has higher fatty acid content (Uchikawa et al. 1986; Ruhland and Fiedler, 1987).

Listeria monocytogenes proteome comprises a total 2853 predicted proteins of which 133 proteins are found on the cell surface. These proteins are either covalently anchored in the peptidoglycans (e.g. InlA), non-covalently attached to the cell wall (e.g. InlB), bound to the cell membrane (e.g. ActA and lipoproteins) or are secreted in the medium. Cell surface proteins aid in colonization, growth, motility, host invasion,

pathogenesis and number of other functions, making *L. monocytogenes* a successful pathogen in foods and food processing environments.

Further below the surface, the cell membrane is made of fatty acids especially the branched chain saturated fatty acids (15:0 and 17:0 iso and anteiso) providing structure and fluidity. The fatty acid composition is altered when bacteria is subjected to food processing stresses like salt, temperature, pH, preservatives etc. thus increasing their survival.

These cellular components along with the genomic make-up of various *L. monocytogenes* serotypes make them unique to niche distribution, survival, stress responses, and pathogenicity. Such information can be helpful in designing various intervention strategies or combinations of hurdles to reduce and/ or eliminate *L. monocytogenes* from RTE foods.

PERSISTENCE OF *LISTERIA MONOCYTOGENES* IN FOOD PROCESSING ENVIRONMENT

Although infrequent, chickens are introduced to hemolytic and non-hemolytic *Listeria* contamination at the farm and can settle on the skin of chicken; mainly on legs. Once in the processing environment, *Listeria* from the chicken can contaminate the processing equipment to form a significant source of *Listeria* cross-contamination. Although scalders and chillers do not contribute to *Listeria* contamination; processing equipment like conveyor belts, tables and sectioning saws can get repeatedly contaminated during the slaughter operation and act as an important source to contaminate chicken parts such as drumsticks, wings, and breasts (Franco et al. 1995; Berrang et al. 2000). These contaminated parts act as a source of contamination in further

processing plants. Nesbakken et al. (1996) found that *L. monocytogenes* isolated from poultry processing plants belonging to electrophoretic pattern B were minor contaminants in cold cuts and dried sausages, concluding that meat is an insignificant source of *L. monocytogenes* (Lunden et al. 2003) compared to worker and general hygiene. Kerr et al. (1993) isolated *Listeria* spp. and *L. monocytogenes* from workers hands handling RTE meat and poultry products and emphasized the need that workers wash their hands adequately or else they can be a source of contamination. In contrast, Berrang et al. (2002) isolated exactly similar strains from drains on raw side of a processing plant and from heat treated products indicating the role of raw materials as an agent to introduce *L. monocytogenes* in a processing environment.

Poultry processing establishments are maintained at low temperatures and have high moisture along with neutral pH, food contact and non-food contact surfaces with food residues. This provides a perfect environment for *Listeria* spp. to survive and grow (Chasseignaux et al. 2002). Senczek et al. (2004) found that different strains of *L. monocytogenes* persist and colonize different niches of processing environments. They found that PFGE type-B was a dominant persistent strain in samples collected over a two year period from processing rooms (surfaces and drains), equipment, and slicing and packing areas of the raw ham production line. On the other hand, PFGE type-E was associated with meat products, raw meat, intermediate production stages, and final products of raw ham production. The PFGE patterns of *L. monocytogenes* in processing areas were similar to the ones isolated from the product indicating cross-contamination between the products and processing environment. Although, PFGE patterns indicated that *L. monocytogenes* isolated from raw and finished products processing areas were

different, the *SmaI* restriction endonuclease digestion profiles indicated a close relationship between those strains suggesting a probable change in the bacterial cell due to niche specific adaptations. Lunden et al. (2003) conducted a detailed investigation on contamination patterns of persistent and non-persistent *L. monocytogenes* on various food contact and non-contact surfaces in meat and poultry processing plants (Table 1) and reported that *L. monocytogenes* strains from raw material were not able to colonize and were non-persistent suggesting that the *L. monocytogenes* persistent strains were introduced onto separate lines independently, or transferred from one line to the other with equipment or personnel, or splashes from inadequate washing procedures or air. They found that the persistent strains were more prevalent in the post-heat treatment equipment such as spiral freezers, slicers, and dicers etc. indicating the importance of these sources in offering a niche for *L. monocytogenes* to colonize and disseminate on products. The strains identified as persistent in one processing plant were grouped as non-persistent in the other plants showing the complexity in *L. monocytogenes* colonization in processing facilities. Moreover, specific ribotypes or PFGE types cannot be related to foods like fish, meat, poultry, and dairy but can be specific to processing facilities. These "house types" or plant-specific persistent strains might have developed abilities to form biofilms or is a result of differences in construction material of those equipment (Aarnisalo et al. 2003; Arnold and Silvers, 2000). Berrang et al. (2002) reported that *L. monocytogenes* could migrate from drains and floors in the raw area to fully cooked product handling areas. On the other hand, Berrang et al. (2005) reported that 3 of the 4 resident/persistent *L. monocytogenes* strains isolated from poultry processing

environment were also detected in raw product underscoring the importance of raw material as a potential source of *L. monocytogenes*.

In conclusion, it can be stated that raw material and worker hygiene are an important source for the introduction of *L. monocytogenes* in the processing environment. The persistence of *L. monocytogenes* in a particular processing plant and particular surface is completely strain dependent rather than serotype dependent. Investigation into the genetic differences of the prevalent and non-prevalent strains of the same serotype or PFGE groups and their phenotypic expression can provide better understanding of the pathogen.

INTERACTION OF LISTERIA WITH OTHER MICROORGANISMS

Listeria has been isolated from water, soil, vegetation, sewage, animal feeds, farms, foods and food-processing environments (Sauders and Weidmann, 2007). In these environments, *Listeria* co-exists and interacts with other bacteria in the environment, which can have either beneficial or detrimental effect on the survival and growth of the pathogen. Bacteria co-exist by forming biofilms that are a complex organization of single or multiple species of microorganism covered with extracellular polysaccharides (EP) formed in nutrient rich niches. Secretion of EP assists in initial attachment, colonization, and growth of the biofilm by offering resistance to various stresses. *L. monocytogenes* can produce EP and form biofilms on wet floors, food processing equipment like conveyor belts, rubber gaskets, drains, stainless steel components, pipe joints and dead-ends by itself or in combination with other bacteria to protect itself from factors such as desiccation, UV, disinfectants (Poulsen, 1999; Shi and Zhu, 2009; Mattila-Sandholm and Wirtanen, 1992; Gandhi and Chikindas, 2007).

Table 1 *Listeria monocytogenes* contamination sites of processing equipment

Processing Equipment	Contamination Site	Direct/Indirect surface contact with product
Freezer	Spiral conveyor	Yes/—
	Supporting structures	No/likely
	Surfaces	UN
Slicing machine	Blades	Yes/—
	Blade cover	Yes/—
	Control panel	No/likely
	Motor	No/unlikely
	Lubricant	No/unlikely
	Ball-race screw	No/unlikely
	Surfaces	UN
Dicing machine	Blade	Yes/—
	Blade cover	Yes/—
	Surface under blade	No/likely
	Product-remains collector	No/unlikely
Peeling machine	Control panel	No/likely
	Surface under the peeler	No/unlikely
	Surfaces	UN
Weigher (with head system)	Funnel	Yes/—
	Surfaces	UN
Packing machine	Chamber	Yes/—
	Surfaces	UN
Conveyor	Belt	Yes/—
	Supporting structures	No/likely

UN, unknown
Source: Lunden et al. (2003)

Biofilm formation by *L. monocytogenes* is strain specific and that growth in biofilm is different than in planktonic cells (Marsh et al. 2003; Chae and Schraft, 2000; Harvey et al. 2007). *L. monocytogenes* strains belonging to different niches like the environment, animals, food processing (persistent and transient) or clinical specimen have varying biofilm forming ability and can be categorized into strong, medium and weak biofilm (Harvey et al. 2007). Additionally, biofilm forming ability depends on the *L. monocytogenes* serotypes. Borucki et al. (2003) demonstrated that *L. monocytogenes* belonging to division II (1/2 a and 1/2c) had a better biofilm forming ability than division I (4b and 1/2b) which was contrary to Djordjevic et al. (2002) and attributed these differences to strains and methodologies used. Bruhn et al. (2005) demonstrated that there is a growth competition between *L. monocytogenes* belonging to different lineages. *L. monocytogenes* lineage 2 (1/2a) when co-cultured with lineage 1(4b and 1/2b) in BHI showed a higher growth in 24 h at 30°C than the later. Similar observations were made by Pan et al. (2009) who studied the biofilm forming capability of *L. monocytogenes* 1/2a (lineage 2) and 4b (lineage 1) in a mixed culture on stainless steel. They found that 1/2 a dominated the biofilm and was more efficient in biofilm formation but the growth of 4b was not inhibited. On the other hand, they observed synergistic effect of certain 1/2 a strain on significant increase in survival and growth of 4b strain cocktail in a mixed culture biofilm. In addition to the inter-lineage effect, growth and survival of *L. monocytogenes* is also affected by *L. innocua* which is commonly isolated from food processing environments.

Carpentier and Chassaing (2004) demonstrated that *Listeria* shares a complex relationship with the bacteria in a mixed biofilm in food processing environments and

that “house flora” can have an effect on attachment of *L. monocytogenes* on inert surfaces. They isolated 29 bacteria from various niches in a food processing environment and tested each strain in a mixed biofilm with *L. monocytogenes*. They found significant inhibitory effect of *Bacillus* spp., *Pseudomonas fluorescens* and *putida*, *Serratia* spp. and *Staphylococcus sciuri* and *warneri*, while *Kocuria varians*, *S. capitalis*, *Stenotrophomonas maltophilia* and *Comomonas testoteroni* had a positive effect on the growth of *L. monocytogenes* in a biofilm. *Staphylococcus* is a species frequently isolated in dairy environments and its interaction with *L. monocytogenes* in a mixed-biofilm was studied by Rieu et al. (2008). They found that the effect of *L. monocytogenes* EGD-e had varying effect on six biofilm forming *Staphylococcus aureus* strains. One particular strain CIP 53.156 increased the biofilm production by EGD-e and further investigations revealed that CIP 53.156 secretes peptides which change the cell morphology and stimulate the biofilm forming ability of EGD-e facilitating its persistence in food processing environments. Similarly, Bremer et al. (2001) found that *Flavobacterium* spp. can enhance the ability of *L. monocytogenes* to attach to stainless steel surfaces as well as to survive temperatures from 4 to -20°C for longer ($p \leq 0.05$) duration as compared to pure cultures. They found that *Flavobacterium* was the primary colonizer which produced more EPS leading to *L. monocytogenes* attachment and this EPS protected *L. monocytogenes* from desiccation even after *Flavobacterium* spp. had died-off. Similar observations were made by Sashara and Zottola (1993) who demonstrated that the production of EPS by a primary organism like *Pseudomonas fragi* can increase the attachment and microcolony formation by *L. monocytogenes* in flowing systems on inert

surfaces furthermore; emphasizing the importance of interaction between *L. monocytogenes* and other environmental isolates.

On the other hand, there are other microorganisms present in food processing environments which can exert a negative impact on the *L. monocytogenes* growth and survival either by competing for nutrients or secreting detrimental biochemicals. Non-specific interactions between microorganisms, termed as Jameson Effect, due to utilization of nutrients by dominant rapidly growing species can cause suppression of *L. monocytogenes* growth. When co-cultured with *Pseudomonas fluorescens*, *L. monocytogenes* showed a suppressed growth at 4°C in BHI (Buchanan and Bagi, 1999).

OUTBREAKS AND RECALLS RELATED TO *L. MONOCYTOGENES*

Listeria is a post-process contaminant and consumption of *Listeria*-contaminated foods can lead to gastrointestinal illnesses or lethal conditions in immunocompromised individuals. A summary of the outbreaks from 1998-2011 related to consumption of *L. monocytogenes* contaminated foods in different states along with the illnesses and fatalities is presented in Table 2. A total of 31 outbreaks occurred from 1998-2011 leading to a total of 578 illnesses, 230 hospitalizations (excluding the cantaloupe recall) and 79 deaths. Of the 31 outbreaks, 5 occurred in 1999, 4 in 2005 and 2006 while there were no outbreaks in 2004. Foods implicated in these outbreaks belonged to RTE category including hotdogs, deli, turkey slices and cheeses (Mexican style soft cheese). Consumption of these products in private homes (24 of 31 outbreaks) caused majority of listeriosis cases while 6 outbreaks were related to foods (taco/nacho salad, grilled chicken and RTE products) consumed at restaurants. Hospital food (deli and tuna salad) was also implicated in 5 of the 31 outbreaks. The majority of fatal listeriosis cases, with 21 deaths,

occurred in 1998 due to consumption of hotdogs manufactured by Bil Mar Foods, MI. This multistate outbreak spanning 24 states occurred as a result of dust generated during the repair of a refrigeration unit that contaminated the product. A multistate outbreak in 2002 involving hospitals, nursing homes, assisted living facilities and home care. This outbreak was due to consumption of deli meats produced by Pilgrim's Pride, PA and led to 54 illnesses and no hospitalizations. However, 8 deaths indicating the vulnerability of elderly individuals, immunocompromised and infants to listeriosis led to stringent *Listeria* regulations in 2003 and 2005 (CDC - NORS Foodborne Outbreak Online Database).

The significance of *Listeria monocytogenes* outbreaks has prompted the USDA FSIS to issue mandatory/voluntary recalls of foods contaminated with/suspected to be contaminated with *L. monocytogenes*. A list of recalls, total number of recalls and the amount of product recalled is presented in Table 2 and 3. Almost all the products recalled are classified as RTE and dinner entrees. The highest number of recalls occurred from 1998-2002 that also coincide with the highest illnesses, hospitalizations and deaths.

The 30 Million lb recall of RTE hotdogs, bologna, turkey deli, breasts and ham in 1999 was the largest ever recall and involved Thorn Apple Valley, AR whose operations were suspended due to their non-compliance to sanitation requirements that forced the company to file for bankruptcy (http://www.nytimes.com/1999/06/26/business/company-news-ibp-to-buy-thorn-apple-valley-for-112-million.html: Accessed: February, 2012).

The most significant outbreak of multistate listeriosis after 1998 occurred in early September 2011 due to the consumption of contaminated cantaloupe produced by Jensen farms, CO infecting 146 people and causing 30 deaths in 28 states. The deaths primarily

occurred in individuals aged 48-96 and one pregnant woman had a miscarriage. An investigation by the US Food and Drug Administration (FDA) revealed that low levels of *L. monocytogenes* on the cantaloupe growing field could have been introduced in the packing facility. It was also found that the truck carrying cantaloupes to cattle operation could bring in *L. monocytogenes* to the packing facility. The investigators found that water pooled on the floor near equipment and employee walkways that was not easily cleanable. The packing equipment was not easily cleanable and some were used in other agricultural operations. Inability of pre-cooling to take the initial heat off of the cantaloupe and directly subjecting the cantaloupe to coolers could have caused condensation of moisture on the surface leading to colonization and growth of psychrotrophic *L. monocytogenes* (http://www.fda.gov/Food/FoodSafety/ CORENetwork/ucm272372.htm).

Various recalls, fatalities and resulting implementation of a strict “zero tolerance policy” on *L. monocytogenes* contamination of RTE products has shown a decrease in the incidences of the pathogen in RTE foods (Figure 1).

Table 2. *Listeria monocytogenes* outbreaks related to consumption of contaminated foods

Year	State	Location Of Consumption	Total Ill	Total Hospi-taliza-tions	Total Deat-hs	Food Vehicle
1998	Colorado	Private home	4			hot dog, unspecified
1998	Multistate	Grocery store; Private home; Restaurant - other or unknown type	101	101	21	hot dog, unspecified
1999	Multistate	Other; Private home	11			pate, unspecified
1999	Florida	Private home	2	2	1	deli meat, sliced ham; deli meat, sliced roast beef; deli meat, sliced turkey
1999	New York	Hospital	6			
1999	New York	Private home	4			hot dog, unspecified
1999	Minnesota	Private home	5	5	1	deli meat, unspecified
2000	Multistate	Other	29	29	7	deli meat, sliced turkey
2000	North Carolina	Private home	12			queso fresco, unspecified
2001	California	Other	56	1	0	potato salad
2001	California	Private home	28	0	0	sandwich, deli
2002	Multistate	Grocery store; Hospital; Nursing home, assisted living facility, home care; Private home; Restaurant - other or unknown type	54		8	deli meat, sliced turkey
2003	Texas	Private home	12	12	1	queso fresco, unpasteurized
2003	New York	Unknown or undetermined	3			
2005	New York	Private home; Restaurant - other or unknown type	6	6	0	

2005	Multistate	Hospital; Private home; Restaurant - other or unknown type	13	13	1	deli meat, sliced turkey
2005	Texas	Private home	12	12	0	queso fresco, unpasteurized
2005	New York	Grocery store; Private home; Restaurant - other or unknown type	3	3	0	chicken, grilled
2006	Oregon	Grocery store; Private home	3	2	1	other cheese, pasteurized
2006	Oregon		2	1	1	
2006	Minnesota	Restaurant - other or unknown type	2	0	0	taco or nacho salad
2006	Ohio	Private home	3			ham, unspecified
2007	Massachusetts	Private home	5	5	3	other milk, pasteurized; skim milk, pasteurized
2008	New York	Hospital	5	5	3	tuna salad
2008	Multistate	Grocery store; Private home	20	16	0	sprouts
2008	Multistate	Private home	8	4	0	cheese, Mexican-style, pasteurized
2009	Multistate	Private Home	8	3	0	mexican style cheese
2009	Illinois	Private Home	6	1	0	
2009	Washington	Private Home	2	2	0	cheese
2010	Louisiana	Private Home	14	7	2	hog head cheese/sausages
2011	Multistate	Private Home	146	?	30	cantaloupe

*Data Source (1998-2009): CDC - NORS Foodborne Outbreak Online Database (FOOD); MMWR 2010; http://www.cdc.gov/*Listeria*/outbreaks/cantaloupes-jensen-farms/100711/index.html

Table 3. List of *Listeria monocytogenes* related FSIS Class 1 food recall incidences and volume from 1994-2010

Year of Recall	Total Recalls	Recall Volume (1000'lbs)
1994	15	26.9
1995	11	24.951
1996	6	4.627
1997	3	18.288
1998	6	36739.949
1999	31	37445.085
2000	35	26181.889
2001	23	14851.275
2002	39	32515.094
2003	15	55.561
2004	14	508.425
2005	29	3449.1
2006	6	48.346
2007	8	2979.233
2008	15	349.661
2009	8	47.341
2010	7	381.526

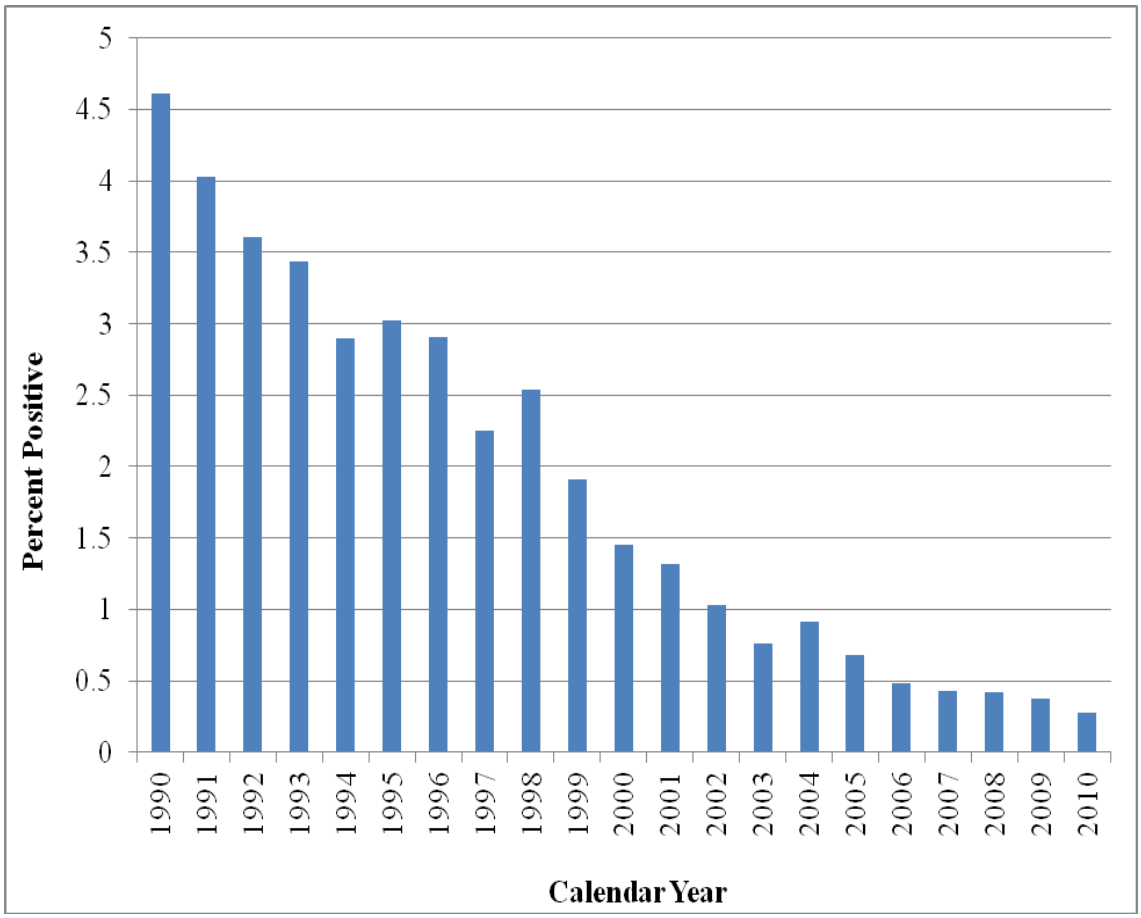


Figure 1. *Listeria monocytogenes* positive (% of the total) ready-to-eat products tested by FSIS from 1990-2010 (Source: http://www.fsis.usda.gov/science/micro_testing_rte/index.asp)

LISTERIA MONOCYTOGENES CONTROL PROGRAM

I. Plant design and construction

Reduction in *L. monocytogenes* contamination can be achieved by compartmentalizing the processing plant into separate finished and raw product areas, raw materials storage, equipment washing facilities, microbiological labs, maintenance areas, offices, and toilet facilities. Lunden et al. (2003) and Keto-Timonen et al. (2007) observed that the RTE processing plants with the most compartments between raw areas, ovens, coolers and slicers, packaging machine and finished product processing lines had the least contamination with non-persistent strains as compared to the intermediately compartmentalized followed by least compartmentalized lines. In addition to raw materials, air-flow can also be a source of *L. monocytogenes* dissemination. Spurlock and Zottola (1991) demonstrated that *L. monocytogenes* can survive aerosolization and potentially contaminate foods and surfaces inside the processing plants. Although, several researchers have found that air or air handling system are not significant sources of *L. monocytogenes* spread (Byrne et al. 2008; Berrang et al. 2010), the USDA guidelines recommend processors to direct the air in a linear flow from finished product to raw product area. Positive air pressure should be maintained in the finished product area, whereas a negative air flow is recommended in the raw product area. Air inlets should be fitted with filters to have an efficiency of 90-95% at 1 micron. Air intake should not be located near the air exhaust and air-make up at the finished product area should be filtered through high efficiency particle attenuation (HEPA) filters (99.97-99.99% at 0.3 micron) to remove bacteria, yeasts and molds.

Water is a major carrier of *L. monocytogenes* if not properly treated and handled (Eklund et al. 1995). Water supply lines for treated and untreated water should be separate and care should be taken to avoid cross contamination. Sewer lines should not be located above the clean water lines neither should they be located above exposed food, food contact surfaces, and food packaging materials. Separate washing areas should be designated for raw and finished product equipment washing (Kirby et al. 2003).

Drains are a major source of *Listeria* contamination since they provide a wet protective nutrient rich environment and can carry *L. monocytogenes* from the raw processing area to the finished product side (Berrang et al. 2005). Drains should flow from the finished product side to the raw side, should be closed and efficient to prevent the assimilation of standing water in and around the drain and should be accessible for cleaning (Tompkin et al. 1999).

The RTE product areas should be equipped with dehumidifying cooling units and drip pans for handling condensation. Ceiling, floors and walls should be smooth, sealed and moisture free; light fixtures should be designed not to harbor dirt or moisture. Any difficult-to-clean overhead light fixtures should be removed from areas where RTE products are exposed. Windows should not be able to open in the finished product areas, wood construction materials should be avoided in all area areas and other wet processing areas in the facility to prevent microbial harborage and cross contamination (Tompkin et al. 1999).

II. Equipment design, construction and maintenance

All processing equipment should be easy to clean possible clean-in-place (CIP). Food contact surfaces should be smooth, non-absorbant, sealed, easily cleanable, sloped

to drains freely, and made of durable non-corrosive and non-toxic materials such as stainless steel. Equipment should not have blunt ends, folds, cracks, open seams, cotter pins, exposed threads and piano hinges. Catwalks and stairs should not be positioned over exposed food or food contact surfaces and constructed such that water does not accumulate in them. Food contact surfaces should be elevated sufficiently above the floor to prevent contamination from food splash. Racks used in the processing facility should have cleanable cover guards over the wheels to prevent contamination of the food from wheel spray.

Equipment maintenance programs should be designed to minimize breakdowns and prevent contamination should repair of equipment be necessary. Scheduled examinations and maintenance of equipments should be done according to the plan. The tools used for raw and finished areas should be completely separate and should not be exchanged between those areas. Equipment that breaks down, and need repairs should be cleaned and sanitized prior to use (Thompkin et al. 1999).

III. Sanitation

A written sanitation standard operating procedure (SSOP) including a sanitation maintenance schedule should be in place for food areas and food contact surfaces and should be strictly followed. Cleaning and sanitizing should include the following steps: (1) remove heavy debris from floors with brooms or shovels, and from the equipment, if needed, (2) pre-rinse the equipment, (3) foam and scrub the equipment with an effective cleaner, (4) rinse the equipment, (5) clean debris from floor, (6) rinse floor with water using a low pressure/low volume hose, (7) use a dedicated brush or floor scrubber to scrub floor with an effective cleaner applying water as needed, (8) thoroughly rinse floors

using a low pressure/low volume hose, (9) sanitize the equipment and floors, and (10) remove excess water from floors if needed (Henning and Cutter, 2001; Thompkin et al. 1999).

IV. Personnel hygiene

Personnel working at the food processing facility can bring *L. monocytogenes* from the environment and contaminate the facility (Kerr et al. 1993). Workers handling raw meats have been shown to carry *L. monocytogenes* on their gloves (Franco et al. 1995). Moreover, Bojsen-Moller (1972) observed that 4.8% of slaughterhouse personnel were carriers of *L. monocytogenes* as opposed to <1% of general population (Jensen, 1993). Similarly, Schuchat et al. (1991) found 11.9% of office personnel and 13.3% slaughterhouse workers were fecal carriers of *L. monocytogenes*. Isolation of *L. monocytogenes* from personnel involved in food processing warrants the need to train workers to maintain hygiene. Practices like washing hands before entering the food production areas, handling exposed foods, food contact areas or packaging material only with gloved hands, using footbaths with sanitizer before entering the processing hall, restricting workers from the raw side from entering the cooked side can help in reducing the transfer of *L. monocytogenes* from workers (Thompkin et al. 1999).

V. Food-contact and non-food contact surfaces/areas

Food contact and non-contact surfaces form a very important niche for *L. monocytogenes* to grow and colonize. These sites provide a wet, protective nutrient rich environment for the pathogen. Sanitation of these surfaces (Table 4 and 5) is critical to control the spread of *L. monocytogenes* inside the processing facility and ultimately on

the foods. The food contact and non-contact surfaces can be divided into primary, secondary, and tertiary sites (Table 4).

Primary site: Product contact surfaces or areas are the primary sites for contamination of product with *Listeria*. A positive contact surface test for *L. monocytogenes* implies that the finished product has touched that area and may be contaminated with *L. monocytogenes*. If any product from the sampled lot has been shipped, the processor should recall the product.

Food contact surfaces contaminated with *Listeria* should be dealt with according to the guidelines given by the USDA (Source: Guidance for Industry: Control of *Listeria monocytogenes* in Refrigerated or Frozen Ready-To-Eat Foods http://www.fda.gov/food/guidancecomplianceregulatoryinformation/guidancedocuments/foodprocessinghaccp/ucm073110.htm).

As per the updated compliance guidelines (October 2004) the USDA states the following:

For repeated contact surface positives, the establishment should also conduct a comprehensive investigation to determine the cause and source of the contamination. This establishment should:

a. Review the cleaning and sanitizing procedures, including the types of cleaning agents.

b. Review traffic control patterns, equipment layout and adherence to employee hygiene procedures.

c. Locate niches

i. Repeated, non-consecutive positives usually indicate the presence of a niche or harborage site for L. monocytogenes

ii. Increase testing of the positive site including individual pieces of equipment to locate the source of the contamination

d. Thoroughly clean and sanitize the individual parts.

i. Intense scrubbing is necessary to breakup or dislodge a biofilm.

ii. A change of cleaning or sanitizing solutions may be indicated.

iii. Fogging of the equipment or room with a sanitizer such as quaternary ammonium compounds could be used if problems persist.

e. Reassemble and test again during operation until the FCS test negative on consecutive tests.

Secondary site: Areas or surfaces in the food processing environment which are not in contact with the product (Table 4) but are close to the direct contact areas and can be a niche for the growth of bacteria. These areas/surfaces can lead to contamination of the above product contact surfaces or the food. By controlling the presence of *L. monocytogenes* in the environment it is possible to reduce the risk of product contamination or product contact surface contamination. If positive samples are found from non-product contact surfaces, follow-up actions should be taken, and may include thorough cleaning of suspect areas and equipment with subsequent intensified/expanded testing.

Tertiary site: *L. monocytogenes* has often been isolated from niches which are either hard to reach or difficult to clean (Table 4). *L. monocytogenes* colonies in such areas/surfaces are likely to contaminate the adjoining environment and eventually the product.

As stated earlier the occurrence of *Listeria* spp. or *L. monocytogenes* on non-food contact surfaces (secondary and tertiary sites) indicates inadequate cleaning and sanitation. If a positive is detected additional sampling should be conducted to determine whether the positive was an isolated incidence. If no additional positives occur, then routine monitoring should be resumed. If re-sampling yield a positive result then the current *L. monocytogenes* control program should be revised, Good Manufacturing Practices (GMP) should be revised, sanitizers should be changed and sanitation should be intensified and employees should be retrained. In the case of repeated positives of non-food contact surfaces, adjoining food-contact surfaces should be sampled. A positive result for *Listeria* spp. or *L. monocytogenes* which was negative earlier indicates that bacteria have migrated from non-contact surface to the contact surface. In this case steps given in primary site section above should be followed.

The flow chart (Figure 2) gives the scenarios on changing sampling plans as per the results for *Listeria* or *L. monocytogenes*.

Table 4. Potential food contact and non-food contact surfaces harboring *L. monocytogenes*

Primary surfaces	**Secondary surfaces**	**Tertiary surfaces**
Brining solutions and injection equipment;	Equipment framework and other equipment in the area	Hollow rollers
Slicing equipment	In-floor weighing scales	Drains
Packaging equipment;	Condensate	Floor
Conveyors	Insulation in walls or around pipes and cooling units that has become wet	Walls
Water or ice used in processing/storage;	Trolleys, forklifts, walk-alongs	Ceilings
Racks for transporting finished product	Cleaning tools such as sponges, brushes, floor scrubbers	Damp Insulations
Spiral freezers/blast freezers;	Maintenance tools	Motor housing
Containers used for holding a food until processing or packaging		Condensate drip pan

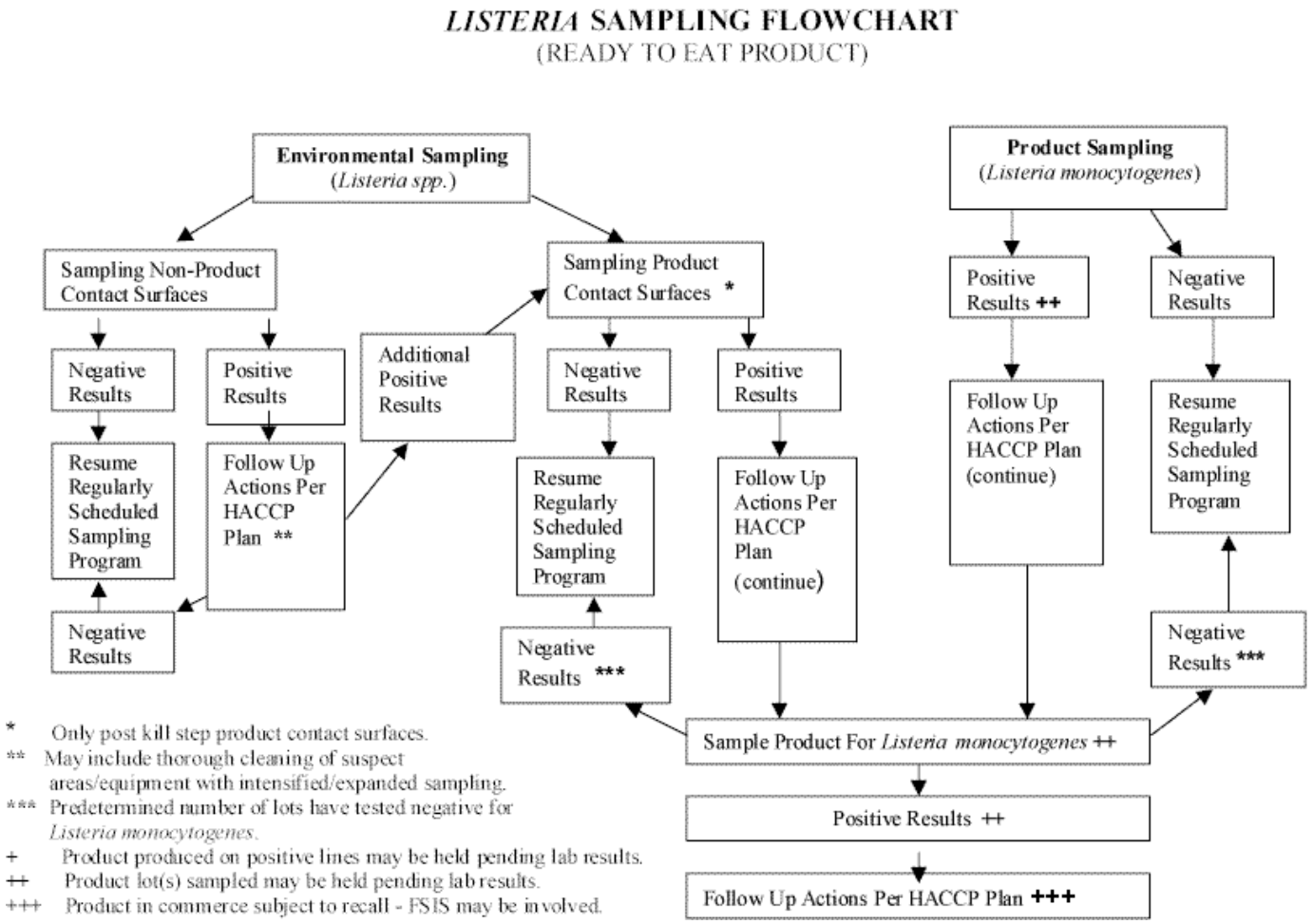


Figure 2. Sampling plan to detect *L. monocytogenes* for a ready-to- eat processing facility Source:

http://www.fsis.usda.gov/oa/ topics/lmguide.htm

Table 5. Recommended frequency for cleaning and sanitizing

Area	**Frequency**
All Processing Equipment	Daily
Floors/Drains	Daily
Waste Containers	Daily
Storage Areas	Daily
Wall	Weekly
Condensate drip pans	Weekly/monthly
Coolers	Weekly/monthly
Freezers	Semiannually

Source: Henning and Cutter (2001)

VI. Controlling *L. monocytogenes* in ready-to-eat foods

The Food Safety and Inspection Service (FSIS) division of the United States Department of Agriculture published 9 CFR 430 in 2003 aimed at controlling *Listeria monocytogenes* in post-lethality exposed RTE meat and poultry products (USDA-FSIS 2011a). The RTE products containing *L. monocytogenes* or which come in contact with surfaces contaminated with *L. monocytogenes* are termed adulterated by the FSIS. The agency states that a RTE manufacturer should control post-lethality exposure of RTE products to *L. monocytogenes* through a Hazard Analysis and Critical Control Point (HACCP) plan or prevent the adulteration by implementing Standard Sanitary Operating Procedures (SSOPs) or other pre-requisite programs. The RTE manufacturer must comply with one of the three alternatives given by the FSIS (Rule 430.4: Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products). Based on the alternatives stated below a RTE product manufacturer might face verification burden by the FSIS.

Alternative 1. An establishment producing RTE products should use an antimicrobial agent or a process to suppress/ limit the growth of *L. monocytogenes* in addition to a post-lethality treatment to reduce and/or eliminate microorganisms on the product.

Alternative 2. An establishment producing RTE products can use either a post lethality treatment to reduce/eliminate microorganisms on the product or an antimicrobial agent/process to suppress/limit the growth of *L. monocytogenes*. Such establishments must maintain sanitation in post-lethality environment (Part 416 –SSOP) since antimicrobials alone are not effective at highest level of contamination.

Alternative 3. An establishment uses only sanitation measures to control *L. monocytogenes* in RTE products.

Establishments choosing Alternative 1 and 2 are recommended (by FSIS) to validate and include the post-lethality step in their HACCP plan while the antimicrobial agent/process to suppress/limit the growth of *L. monocytogenes* can be included in the HACCP or SSOP or other prerequisite program. Post-lethality steps include but are not limited to steam/hot water pasteurization, pre-packaged/post-package surface pasteurization, high hydrostatic pressure processing, ozone and organic acids. Establishments choosing Alternative 2 and 3 should test food contact surfaces to ensure sanitary conditions for control of *L. monocytogenes* in post-lethality processing environments. The USDA-FSIS requires such establishments to have a plan ready for hold-and-test scenario wherein the food contact surface tests positive for *L. monocytogenes*. These establishments should state the size, location and frequency of sampling along with justification of sampling frequency. Sampling frequencies must be justified and should be based on history and trends of *L. monocytogenes* occurrence in a plant, features of the plant, type of product and volume, plant layout and product flow. In addition, establishments choosing alternative 3 producing hotdog or deli products must verify corrective actions taken in case of *L. monocytogenes* positive food contact surfaces, test and hold a product when a second positive is found and sample and test lots to release or rework them to destroy *L. monocytogenes*. Reduction/elimination of *L. monocytogenes* in alternative 3 is completely dependent on sanitation programs and is subjected to more frequent verification testing by FSIS followed by the alternative 2 and 1 establishments.

Implementation of 9 CFR 430 in 2003 significantly reduced occurrence of *L. monocytogenes* in RTE products possibly because of strengthened control procedures, improved *Listeria* interventions and increased sampling. Results from FSIS testing for *L. monocytogenes* in RTE products indicate that percent *L. monocytogenes* positive samples declined from 4.61% in 1990 to 0.28% in 2010 (USDA-FSIS 2004, http://www.fsis.usda.gov/science/Micro_Testing_RTE/index.asp#results10). Reduction in *L. monocytogenes* is reflected in the 38% lower incidence of the pathogen in 2010 compared to 1996-1998 FoodNet data (MMWR, 2011). Similarly, the baseline data of "Healthy People" for *L. monocytogenes* showed decreased incidences per 100, 000 from 0.47 case rate in 1996 to 0.30 in 2010.

INTERVENTION STRATEGIES

Significant research has been conducted on the use of various strategies to eliminate *L. monocytogenes*. The most common and effective intervention strategies include chemical additives like lactates and diacetates at or below the permissible levels. The major disadvantage of using chemical additives is that they do not follow the current market trends of "all natural" and "clean label". Processors are faced with the dilemma of conforming to a "zero tolerance" for *L. monocytogenes* and the market trends at the same time. Recently, several chemical interventions have proven effective along with physical methods like ultraviolet irradiation and ultrasound. The following review focuses mostly on the non-synthetic chemical and physical intervention strategies, their mode of action and factors affecting their anti-listerial efficiencies.

I. Lactic acid and their salts

Lactic acid and its salts are commonly used as antimicrobials in foods. Sodium and potassium lactates are proven to be effective against *L. monocytogenes* either as ingredients or as topical applications in RTE foods. Although, its antimicrobial mode of action is not completely undestood, it is argued that sodium lactate (NaL) reduces water activity which can potentially effect the pathogen. Moreover it is speculated that lactates reduce intracellular pH leading to impaired cell functions and reducing cell growth (Shelef, 1994). Contrary to the latter assumption, McWilliam Leitch and Stewart (2002) proved that alteration in intracellular pH, proton gradient or membrane potential were not responsible for antimicrobial effect of L-lactate on *E. coli*. Inspite the ambiguities about its mode of action, lactates are being continually used as antilisterial agents in RTE products.

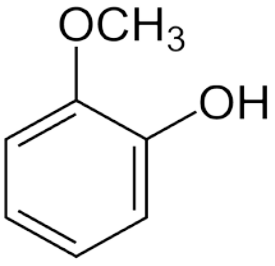
The maximum permissible levels of sodium lactate (NaL) are 3% (4.8% of the commercially {60% wt/wt} available compound). Several researchers have used lactates either alone or in combination with diacetates as ingredients or post-lethality dip in meat products. Bedie et al. (2001) demonstrated that frankfurters formulated with 3% sodium lactate showed a listeriostatic effect until 70 days while at 6% exhibited listericidal activity for 120 days at 4°C. Similarly, Choi and Chin (2003) reported that listeriostatic effect of 3.3% NaL as a sausage ingredient extended the lag phase by at least 2 weeks. On the other hand, Nunez De Gonzalez et al. (2004) reported that frankfurters made with 3.3% of potassium lactate (KL) and then dipped in KL solution did not show any antimicrobial effect against *L. monocytogenes* or spoilage microflora similar to the control samples during 12 weeks of vacuum storage at 4.5°C. Contradictory findings by

Porto et al. (2002) indicated that the use of 2 or 3% KL as an ingredient had a listeriostatic effect on frankfurters inoculated with (either 20 or 500 CFU) *L. monocytogenes* over a 90 day vacuum storage period at 4°C. Knight et al. (2007) reported that a combination of sodium lactate and diacetate treatment with irradiation of frankfurters retarded the growth of *Listeria* from week 6 to 8 during the 8 week storage period. Similarly, Yoon et al. (2004) proposed that addition of sodium lactate and diacetate could enhance safety during refrigerated and frozen storage of smoked salmon.

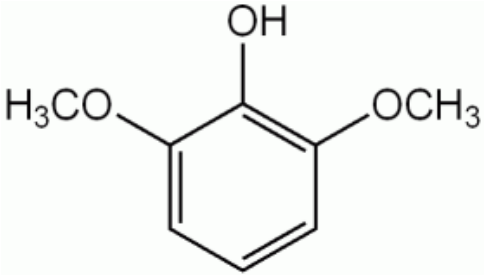
Although, lactic acid and its salts are the most common additives in RTE products; they are synthetic and contradict the current market trends. Some of the alternative non-synthetic/natural antimicrobials discussed below.

II. Smoke and its' components

Smoking is an ancient preservation technique that increased the shelf life of seafood and meats in addition to imparting flavor, color and aroma to the product. Smoke is generated by burning various kinds of woods and comprises of carbonyls, phenol derivatives, furan and pyran derivatives, ketones, diketones, alkyl aryl ethers, aldehydes, pyrocathecol derivatives and hydrocarbons (Guillen et al. 1995). Among these derivatives, phenolic compounds exhibit antilisterial effect as well as inhibit general spoilage microflora (Cornu et al. 2006).



Guaiacol



Syringol

Figure 3. Phenolic compounds such as guaiacol and syringol are a part of wood smoke provide flavor and aroma to smoked products

Source: http://en.wikipedia.org/wiki/Syringol, http://en.wikipedia.org/wiki/Guaiacol

Exposure to phenols, influences the lipid-protein ratio, alters fatty acids isoforms of the membrane giving it rigidity and preventing leakage of cell constituents as well as increasing respiration during growth (Keweloh et al. 1990). Although the mode of action of smoke compounds is not completely elucidated, Guilbaud et al. (2008) used liquid smoke to study the alteration in the proteomic pattern and hemolytic ability of *L. monocytogenes*. They observed a higher antilisterial activity of the smoke extract with an increase in the phenol content reflected by up- and down regulation of proteins respectively. Some of these proteins are involved in glycolytic pathways, metabolism of co-enzymes, prosthetic groups, carbohydrates and nucleotides thus altering the normal cell function. An increase in phenol concentration led to an increase in synthesis of ClpP-2: a serine protease (general stress protein) and virulence factor but there was no change in the expression of haemolysin (*hly*) gene expression (Guilbaud et al. 2008). In addition they observed that the hemolytic potential of *L. monocytogenes* in Lauria Bertani medium decreased 2 and 4 fold after exposure to 3.5 and 7 µg/mL phenol, respectively, as compared to the control and was absent at 15 µg/mL. It has been observed by Faith et al. (1992) that phenolic compounds present in the smoke help in extending the lag phase and generation time, more so when the pH is slightly acidic. These researchers also reported that isoeuginol (a phenolic compound) is highly efficient in increasing the lag period of *L.* Scott A from 8 to 16 h. Another phenolic compound identified as juglone with a double benzene ring which was isolated from teak bark, was found to have antilisterial

properties (Neamatallah et al. 2005). Sunen et al. (2001) found that the smoke extract with the least concentration of phenols was most effective as an antimicrobial, indicating that there are other compounds contributing to the inhibitory effect of smoke. Irrespective of the mode of action, the antibacterial efficiency of smoke depends on the wood source, chemical composition of smoke, bacterial genera/species as well as application, food processing and storage conditions (Asita and Campbell, 1990; Sofos et al. 1988).

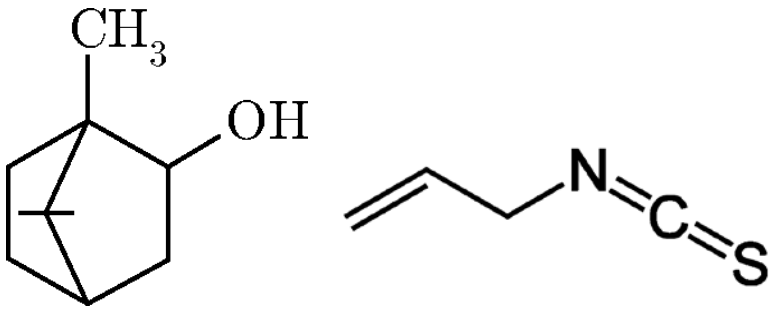
III. Plant extracts

As more and more consumers are demanding removal of synthetic chemical preservatives from foods, the food industry is finding alternatives in natural antimicrobials. These antimicrobials are essentially part of the spices and herbs being used in various culinary traditions around the world for centuries. In addition, novel antimicrobial compounds are being identified from plants occurring in various parts of the world. The following is a review of some known natural antimicrobial compounds, their modes of action and use against *L. monocytogenes*.

Plant extracts can be obtained from the flowers, fruits, barks, leaves, stem and even roots and include essential volatile oils and secondary metabolites (alkaloids, flavonols, flavonoids and polyacetylenes) composed of phenolic and non-phenolic active components. Antimicrobial compounds have been extracted from spices like cilantro, rosemary, sage, oregano, clove, thyme and horse radish to name a few.

Phenolic compounds, carvacrol and thymol affect the integrity of the cell by solubilizing lipopolysaccharides, disrupting the outer cell membrane, leading to leakage of cell constituents (Halender et al. 1998). Another speculation regarding breakdown of the cell membrane and its function, is that phenols alter the pH gradient and electrical

potential across the membrane by a) changes in ion transport or depolarization by changing membrane fluidity; b) interferance with ATP generation; and/or c) inhibition of enzyme to utilize substrate for energy production (Holley and Patel, 2005).



(A) (B)

Figure 4. Examples of (A) phenolic component- Borneol and (B) non-phenolic component- allyl isothiocynate occurring in plant extracts

Source: http://img.tradekey.com/o-B4597114-20101105095913/sell-borneol.jpg; http://en.wikipedia.org/wiki/File:Allyl-isothiocyanate-2D-skeletal.png

Many studies have been conducted on the use of plant extracts as antilisterial agents. Hao et al. (1998) demonstrated that clove extract (eugenol) was effective in significantly limiting growth of *L. monocytogenes* on refrigerated cooked beef stored at 5°C as compared to the extracts from angelica root, banana puree, bay, caraway seed, carrot root, marjoram, pimento leaf, and thyme. This study indicates that the antimicrobial effect of plant extracts is dependent on the chemical composition of the extract.

VI. Nisin

Nisin, a lantibiotic, is a positively charged 34 polypeptide bacteriocin secreted by *Lactococcus lactis* subsp *lactis* commonly added as an antimicrobial in foods. Antibiotic properties of nisin are attributed to the following:

1) ability to alter the permeability of the lipid bilayer and to make holes causing expulsion of ions and protons, disrupting proton motive forces and uptake of solutes;

2) making large enough holes for diffusion of large solutes;

3) disruption of primary transport system causing efflux of solutes;

4) it affects the secondary transport systems preventing coupling of energy to the proton motive forcecausing efflux;

5) it can cause damage to the cell by any of the above combinations.

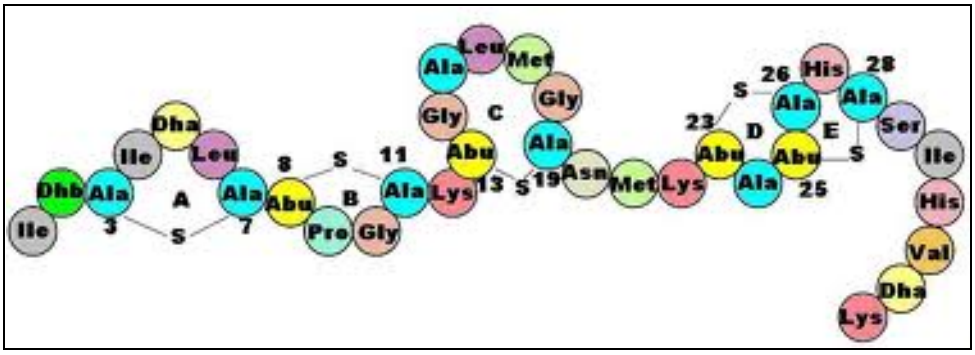


Figure 5. Primary structure depicting the amino acid sequence of nisin

Source: http://hu.wikipedia.org/wiki/Fájl:Nisin_2d_amino_structure.JPG

Chung et al. (1989) determined that *L. monocytogenes* was highly susceptible to nisin and was reduced from 7 logs/mL to less than 10/mL in 30 s in presence of 10^4 IU/mL nisin. Moreover they found that application of nisin on meat can significantly affect bacterial attachment and growth at 5°C. Budu-Amoako et al. (1999) suggested that addition of nisin to cans will help in reducing the heat process of cold-packed lobster meat. Boziaris and Nychas (2006) modeled the growth of *L. monocytogenes* Scott A at temperatures from 5-35°C at different water activities, pH, salt concentrations and nisin. They found that although *L. monocytogenes* Scott A could grow at pH 4.81 in absence of nisin; addition of nisin increased the pH and water activity needed for growth. They also

noticed that addition of 2.5-4.5% NaCl (w/v) increased the resistance of *L. monocytogenes* Scott A to nisin. Similarly, Thomas and Wimpenny (1996) reported that nisin resistance increased at pH 4.5 to 5.

Although nisin is an effective antimicrobial, there are concerns about the increase in resistance by pathogens like *L. monocytogenes*. Nisin resistant *L.* Scott A strains exhibit a higher percentage ($p>0.05$) of straight chain saturated and unsaturated fatty acids compared to branched-chain at low temperatures causing increased fluidity compensated by change in C15/C17 ratio providing increased rigidity to prevent nisin insertion in the membrane. The resistant strains show a higher phosphatydylglycerol and lower diphosphatidylglycerol compared to the wild type (Mazzotta and Montville, 1997; Verheul et al. 1997).

V. Ultraviolet light

Ultraviolet light has shown to exhibit microbicidal properties between 250-270 nm (UVC) (Guerrero-Beltran and Barbosa-Canovas, 2004). The microbicidal activity of UV is due to its ability to induce formation of cyclobutane pyrimidine dimers in genomic DNA (Eischeid and Linden, 2007). These dimers interfere with DNA processing and affect cell physiology leading to cell death.

Due to its ease of application and effectiveness as a non-thermal bactericidal technique, UV has the potential in food disinfection system (Guerrero-Beltran and Barbosa-Canovas, 2004). Since UVC lacks capability to penetrate, it is generally used for surface disinfection (Guerrero-Beltran and Barbosa-Canovas, 2004), and has been applied to disinfect various surfaces like chicken skin (Sumner et al. 1996), skinless chicken breast meat (Lyon et al. 2007), eggs (Coufal et al. 2003), packaging material

(Silva et al. 2003) to name a few. The effectiveness of UVC (254nm generally used) depends on:

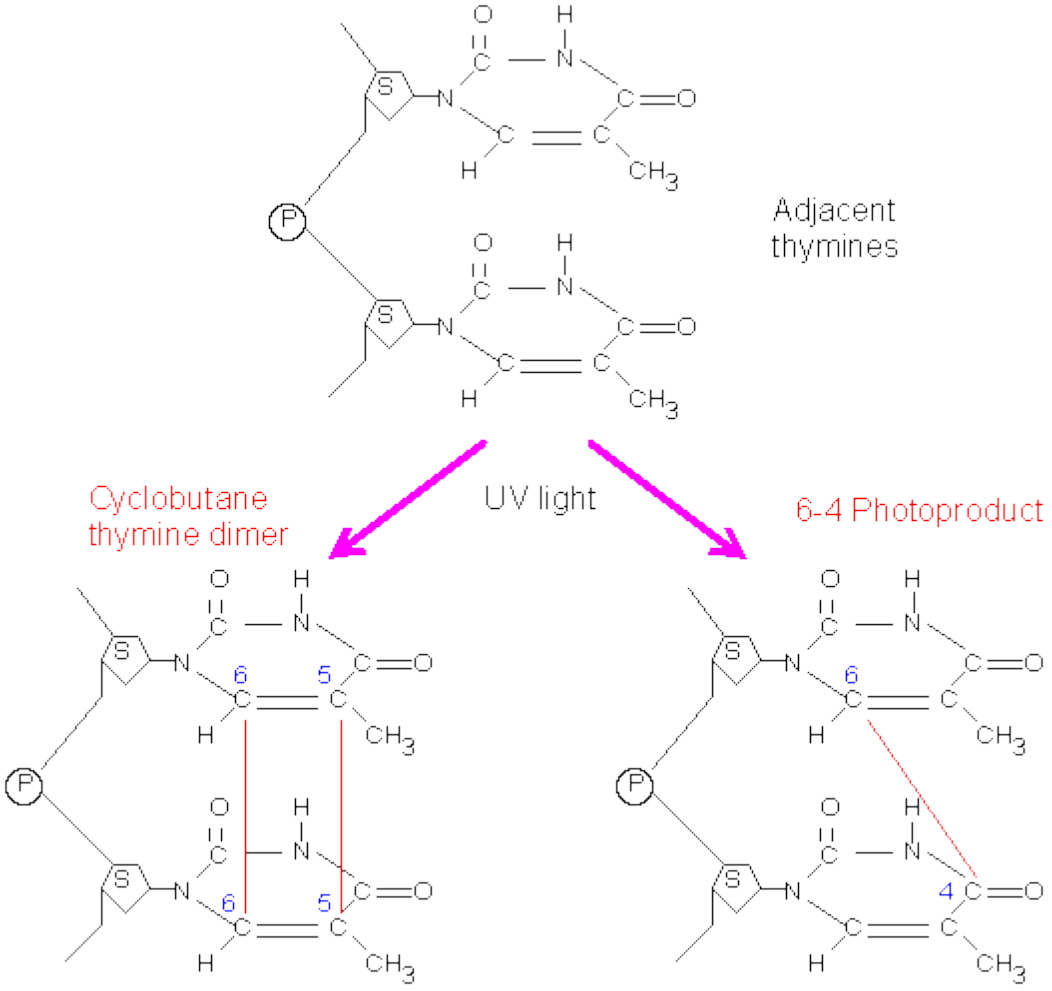


Figure 6. Formation of pyrimidine dimers cellular DNA after exposure to UV light

Source: http://www.web-books.com/MoBio/Free/Ch7F5.htm

1. **Surface or medium exposed**: Smooth surfaces like stainless steel (Kim et al. 2002) and low density polyethylene (Silva et al. 2003) have greater efficiency of reducing bacterial load as opposed to rough perforated chicken skin (Sumner et al. 1996). Ozer and Demirci (2006) reported a difference in reduction of *Escherichia. coli* O157:H7 and *L. monocytogenes* on skin and muscle side of salmon exposed to UVC radiation at 5.6 J/cm^2. UVC does not have a high penetration capability, therefore, cracks and

crevices on the surface can provide a shelter to bacteria thus reducing the radiation efficiency (Silva et al. 2003).

2. **Target bacteria**: The differences in UV sensitivities of bacteria are well documented in literature. Allende et al. (2006) reported that *Salmonella* Typhimurium along with three other bacteria were more resistant to UV compared to 16 other bacteria isolated from "Red Oak Leaf' lettuce. On the other hand Coufal et al. (2003) observed only 1-2 log reduction difference between *S.* Typhimurium and *E. coli* inoculated on egg surface and exposed to 4-14 mW/cm^2 UVC. Lyon et al. (2007) did not observe major differences in reduction of 4 Subtypes of *Listeria monocytogenes* inoculated on boneless, skinless breast fillets exposed to 1000 uW/cm^2 UVC for 5 min. Bacterial concentration and growth phase can also affect UV efficiency.
3. **Energy intensity and time of exposure**: Kim et al. (2002) exposed *E. coli* O157:H7, *L. monocytogenes* and *S.* Typhimurium to UVC at 250 uW/cm^2 and 500 uW/cm^2 for 1, 2 and 3 minutes. They observed that in longer exposure of all the bacteria in peptone water at 250 uW/cm^2 is more efficient than shorter at 500 uW/cm^2. On the contrary they did not find such a difference when bacteria were inoculated on stainless steel chip.

Stermer et al. (1987) showed that UV was effective in reducing the bacterial counts on smooth meat surface as opposed to rough cuts and that organoleptic scores of irradiated and non-irradiated meat samples were not different ($p>0.05$) during refrigerated storage. They concluded that UV can increase the lag phase thus enhancing the shelf life of meat. Similarly Sumner et al. (1996) showed a maximum reduction of 90% of *S.* Typhimurium on UV treated chicken carcass and concluded that the use of UV

along with additional antimicrobial interventions can reduce the bacterial load on chicken. Sommers et al. (2009) found no change in appearance and odor of frankfurters after UV treatment but reduced the *L. monocytogenes* counts by 2 logs. Huang and Toledo (1982) demonstrated the use of UV in reducing the initial bacterial load on fish and thus increasing the shelf life of fish during chill storage. Ozer and Demirci (2006) also found a 90% reduction in *E. coli* and *L. monocytogenes* counts on salmon fillets after treatment with pulsed UV light. Lyon et al. (2007) stated that the use of UV in reducing *L. monocytogenes* on raw poultry could be used as an effective tool to reduce transmission of *L. monocytogenes* to poultry further-processing facilities. Most of the literature is on the use of UV on raw poultry/meat/fish but USDA-FSIS data indicates *L. monocytogenes* as a major contributor to the recall of RTE poultry products. Hence it is important to conduct research on effectiveness of UV as an intervention step to reduce pathogen load on processed RTE products.

Ultraviolet light has been used as a preservation technique in various foods. Although, UVC is effective against bacteria it is important to study the development of resistance to food processing parameters like pH and salinity.

Exposure of bacteria to UV leads to damage in the chromosomes. The dimers formed in the genomic DNA by UV are excised by the DNA repair mechanism. The nucleotide excision repair (NER) mechanism involves *uvrABC* complex. *uvrA* has also been associated with an increased survival of acid adapted *L. monocytogenes* strains when subjected to UV as compared to non-adapted *L. monocytogenes*. Hence it has been proposed that UV-resistance may induce increased pH tolerance. The acid tolerance

response of *Listeria* has shown to induce cross-resistance to other stresses like temperature and salt concentration.

Most of the stress-adaptation and cross-resistance studies are conducted in defined medium that are different from the actual food system. The growth patterns of bacteria in a defined medium are different when compared to the food system. It will be of interest to compare the growth patterns of *L. monocytogenes* strains under defined conditions as well as a model food system. Such data of growth patterns can lead to development of mathematical models for UV tolerant bacterial strains. These models can help food processors using UV technology to keep *Listeria* contamination under control.

VI. Microwave

Microwaves are used frequently for heating/reconstituting foods. Heating due to microwaves is caused by molecular friction between the dipolar rotation of polar solvents like water and conductive migration of dissolved ions inducing injury or death of bacteria in those foods (Heddleson and Doores, 1994; Farber et al. 1998). The lethality of microwaves depends on type and load of target bacteria, as well as different products. Uniformity of temperature within microwaved foods as well as inadequate cooking/reheating can lead to further food safety concerns (Heddleson et al. 1996). The efficiency of microwaves to heat foods uniformly depends on (Oliveira and Franca, 2002):

1. **Type of food**: (product density and constituents)
2. **Chemical composition of foods**: Dielectric properties of foods are mainly affected by the moisture and ionic content of food. Increase in ionic concentration results in

increased power absorption due to the increased dissipation of energy resulting in higher temperatures compared to low ionic content samples.

3. **Size and shape**: The absorption of microwaves decreases exponentially with increased depth of samples. In smaller samples, heating is uniform throughout while surface heating is observed in large samples. Asymmetric shapes lead to non-uniform temperature but can be reduced by rotation of samples.
4. **Temperature of food**: Microwave power absorption is reduced significantly when water is frozen and can lead to higher temperatures in thawed regions as opposed to frozen sections of the same food.
5. **Frequency of microwaves**: Lower frequencies (915 MHz) heat samples more effectively than higher frequencies (2450 MHz) due to more efficient dissipation of electromagnetic energy into heat. In general higher frequencies are used to allow better heating control.

VII. Ultrasound

Sound above the range of normal human hearing of 20 kHz is generally termed as ultrasound and has found potential application in food processing and preservation. Ultrasound waves can be classified into low (<1 W/cm) or high intensity (100-1000 W/cm) ultrasound depending on its application. Low intensity ultrasound is a non-destructive way for analyzing foods without causing any alterations in food composition. On the other hand, high intensity ultrasound can be employed for surface decontamination, denature enzymes, extraction of organic compounds, and crystallization during freezing and modification of meats (Mason et al. 1996; McClements, 1995).

Unlike microwaves, ultrasound produces bubbles or cavities in foods that become bigger with time and vibrate and finally collapse producing high pressure (5 Mpa) and temperature (5,500°C) enough to damage or disrupt bacterial cell wall leading to cell death. Moreover, free OH^- and H+ radicals formed during sonolysis of water degrade cellular DNA causing disruption of normal cell function. Stress-response studies on *Escherichia coli* indicate that sublethal ultrasound exposure cells trigger high heat shock, membrane damage, oxidative stress and SOS response. The effectiveness of ultrasound depends on the temperature of the medium, frequency, viscosity of the medium along with the bacterial species and other interventions strategies applied for food preservation. (Earnshaw et al. 1995; Mason et al. 1996, Vollmer et al. 1998).

Ultrasound is more effective in lower viscosity foods like juices and milk since the medium allows higher degree of cavitation resulting into increased bactericidal activity. Inactivation of *L. monocytogenes* in apple cider treated with ultrasound was 1.64 $\log_{10}$ CFU/mL and 7.85 $\log_{10}$ CFU/mL at 20 and 60°C when compared to 0.16 and 5.48 $\log_{10}$ CFU/mL after respective thermal treatment alone (Baumann et al. 2005). The D-values of thermally treated cider were 117 min at 20°C which reduced to 12.3 min when combined with sonication indicating that thermosonication has additive inactivation effect on *L. monocytogenes*. High temperatures can increase cavitation (until the boiling point) and injure bacteria thus increasing their susceptibility to ultrasonic waves. Similar synergism can be observed by addition of lipohilic phenolic antimicrobials like vanillin and citral which accumulate in lipid bi-layer, weaken the cell wall increasing cell's susceptibility to ultrasound. On the other hand, ultrasound damage to the cell wall

enhances the antimicrobial action of antimicrobials (Baumann et al. 2005; Ferrante et al. 2007).

Lee et al. (2003) did not find any effect of ultrasound at 34.6 W at 30 and 150s on the reduction of *L. seeligeri* in liquid whole eggs at 5°C indicating that ultrasound is more effective at higher temperatures which are not conducive for certain foods. Similarly, Stanley (2004) reported that reduction in cell numbers of *L. monocytogenes* Scott A in salt solutions were higher at 40°C than in ice bath. Moreover it must also be noted that different strains of *L. monocytogenes* have varying sensitivity towards ultrasound inactivation (Baumann et al. 2005). The increased protection of *Listeria* spp. against ultrasound waves is probably due to the thick peptidoglycan layer in the cell wall as compared to the gram-negative bacteria.

Many researchers have attempted application of ultrasound in meats. Birk and Knochel (2009) studied the effect of ultrasound in combination with marinades and temperature on the reduction of *L. monocytogenes*, *Brocothrix thermospacta*, *Carnobacterium maltaromaticum* and *Campylobacter jejuni*. They found that an exposure for 10 min at combined high (1 MHz) and low (25 kHz) at 12°C reduced ($p \leq 0.05$) of all the above bacteria when compared to red wine marinade alone. A similar study was conducted by Hansen and Larsen (2007) who used a patented technology, 'SonoSteam', for surface decontamination of chicken carcasses. SonoSteam uses ultrasound to perturb and remove the laminar boundary layer on the surface of the carcass after which hot steam blown over the carcass kills the surface bacteria. The company claims to reduce 2.5 $\log_{10}$ *Campylobacter* within 10 s without and visual defects on the carcass quality.

Ultrasound has also been used to decontaminate conveyor belts made from different material. A short 30-60 s exposure at 30-45°C reduced *L. monocytogenes* up to 5.95 log units on acetal belts while stainless steel belts showed a reduction of 5.90 log units when used in combination with cleaning detergents (Tolvanen et al. 2007).

Disadvantages:

1. Temperature increase of liquid whole eggs from 5°C to 15°C and 30°C after 30 and 150 s, respectively, after exposure to 34.6 W ultrasound (Lee et al. 2003). The temperature can be detrimental to the products, hence long time low intensity would be beneficial for maintaining product integrity.
2. Time of exposure is very high. Exposures as high as 10 min are required to achieve a desired antimicrobial effect on meats (Birk and Knochel 2009).This exposure can be reduced by using high intensities.
3. Production of free radicals can initiate lipid oxidation (McClements, 1995) thus affecting product shelf-life.

Listeria monocytogenes is a complex food-borne pathogen with unique ability to survive and grow and persist in food processing environments where other bacteria can be easily removed by sanitation. They have been documented to contaminate a wide variety of RTE foods and cause significant human and economy harm. The government has developed and revised regulations to reduce or eliminate *L. monocytogenes* in foods and prevent the losses. Strenuous research is being conducted to find novel intervention strategies to avoid *L. monocytogenes* from farm to fork. In-spite of these efforts, *L. monocytogenes* is a constantly evolving and emerging major pathogen of concern in RTE products.

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CHAPTER III

EFFECT OF LIQUID SMOKE AS AN INGREDIENT IN FRANKFURTERS ON *LISTERIA MONOCYTOGENES* AND QUALITY ATTRIBUTES

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ABSTRACT

Market trends indicate an increased interest in 'natural antimicrobials' to augment safety of ready-to-eat meat and poultry products against *Listeria monocytogenes*. Liquid smoke, an all natural condensate of smoke components, applied as a post-process treatment on the product surface has the potential to exhibit antilisterial properties. Studies on its antimicrobial efficacy and quality attributes as an ingredient are lacking. A study was designed to validate the antimicrobial effect of liquid smoke as an ingredient against *L. monocytogenes* and its impact on the shelf-life and quality of frankfurters.

Chicken and pork frankfurters were incorporated with 0, 2.5, 5 and 10 % liquid smoke (Zesti Smoke ^{TM}). Cooked casing-stripped frankfurters (4 per package) were placed in vacuum pack bags, spray inoculated with either high (8 $\log_{10}$ CFU/ mL) or low (4 $\log_{10}$ CFU/ mL) levels of *L. monocytogenes* serotype 4b, vacuum packaged and stored at 4°C for up to 12 weeks. Sampling was done every week for 12 weeks to estimate growth of *L. monocytogenes* growth, spoilage microflora (aerobic plate counts, yeast and molds, lactic acid bacteria and total coliforms), sensory attributes and texture profile analysis. Data was analyzed to find significant differences at $p \leq 0.05$.

Incorporating smoke extract at 2.5, 5, and 10 % reduced ($p \leq 0.05$) populations of *L. monocytogenes* as compared to the controls throughout the storage period irrespective of the inoculation levels. Furthermore, incorporation of smoke extract did not affect

($p>0.05$) the texture, juiciness, flavor and overall scores as well as hardness and chewiness of the frankfurters. Liquid smoke can be effectively incorporated as an 'all natural' antimicrobial in the manufacture of frankfurters without negatively affecting quality attributes.

Keywords: Liquid smoke, *Listeria monocytogenes*, shelf-life, organoleptic evaluation

INTRODUCTION

Listeria monocytogenes contributes significantly to food related illnesses and deaths in the United States (Scallan et al. 2011). Schraff (2012) estimated that the total cost of illnesses caused due to *L. monocytogenes* in 2010 to be $2.04 billion. These incidences are due to the consumption of *Listeria*-contaminated foods especially by immunocompromised, elderly, new-born and pregnant women. The severity of listeriosis has resulted in the USDA issuing a "zero tolerance" policy for *L. monocytogenes* on ready-to-eat (RTE) products. Elimination of *Listeria* from products and prevention of its growth during storage requires use of interventions such as antimicrobials as ingredients and post-lethality treatments (USDA-FSIS, 2004).

Synthetic chemicals such as lactates and diacetates have been effectively used to reduce and/or eliminate *L. monocytogenes* in RTE meat and poultry products (Bedie et al. 2001; Choi and Chin, 2003). Porto et al. (2002) indicated that the use of 2 or 3% potassium lactate as an ingredient had a listeriostatic effect on frankfurters inoculated with (either 20 or 500 CFU/package) *L. monocytogenes* over a 90 day vacuum storage period at 4°C. Knight et al. (2007) combined a sodium lactate and diacetate treatment with irradiation of frankfurters that resulted in retarding the growth of *Listeria* from week 6 to 8 during the 8 week storage period.

However, Bowers (2008) indicated that sodium lactates and diacetates can reduce water-binding capacity and impart off-flavors thereby compromising product quality. Moreover, these antimicrobial additives must be mentioned on the food label, which is against current market trends of "clean label". Market demands for "all natural" and

"non-synthetic/-artificial antimicrobials" have stimulated the emergence of antimicrobials manufactured from natural sources.

Wood smoke contains antimicrobial compounds and has been used to impart flavor, color and preserve meats. Antimicrobial properties of smoke are due to phenols and carbonyl compounds naturally present in wood (Holley and Patel, 2005). These chemical compounds responsible for antimicrobial properties have been isolated and incorporated in commercial liquid smoke preparations. Commercial liquid smoke like "Zesti Smoke™" (Kerry Ingredients and Flavours, TN), a unique water soluble combination of natural smoke extracts, has the potential to have antilisterial activities. Zesti Smoke™ can be mentioned as "natural extracts" thus following the "clean label" trend simultaneously offering listeriostatic/cidal properties.

Liquid smoke preparations can be either incorporated as an ingredient or topically applied on the surface to reduce and/or eliminate *Listeria* as well as impart a desired smoky flavor to the product. Several studies have been conducted on the use of liquid smoke as a post-lethality dip/spray treatment to reduce/eliminate *Listeria* on RTE products (Milly et al. 2000; Sunen et al. 2001; Vitt et al. 2001; Gedela et al. 2007 a,b). Antimicrobial efficacy of liquid smoke can be enhanced by lowering the pH with organic acids to as low as 2.2 (Faith et al. 1992; Gedela et al. 2007b) and therefore can only be applied on product surface rather than incorporating it as an ingredient. Surface application requires separate equipment, drain time and drip handling thereby delaying packaging process. The potential to remove these hurdles by the use of neutral pH liquid smoke as an ingredient in the RTE products warrants investigation. Therefore, research was conducted over a period of 12 weeks to validate the efficacy of using a neutral pH

liquid smoke as an ingredient at varying concentrations on the growth of *L. monocytogenes* 4b inoculated on hotdogs. Additionally, microbial shelf life, sensory acceptability and changes in physical properties of the hotdogs were also assessed over a period of 12 weeks at 4°C under vacuum.

MATERIAL AND METHODS

Bacterial culture preparation. *Listeria monocytogenes* serotype 4b was cultured in sterile brain heart infusion (BHI, Acumedia, MD) broth (10 mL) to achieve approximately 8 $\log_{10}$ CFU/ml after 18h of incubation at 37ºC. The resulting bacterial culture (0.1 mL) was distributed and grown in three separate BHI tubes (10 mL) for 18h at 37°C and repeated 5 times to obtain three independent bacterial populations. *L. monocytogenes* cells from the final generation were re-cultured in 3 separate BHI (800 mL) bottles for 18h at 37 °C. Individual cultures were centrifuged (Sorvall Legent RT+ Centrifuge, Thermo Scientific, Thermo Electron Corp., Germany) at 1294.3 x g for 20 min at 4°C and the pellets were resuspended in equivalent amounts of sterile peptone water (PW; Acumedia, MD). Lower concentrations (approximately 4 $\log_{10}$ CFU/mL) of *L. monocytogenes* were obtained by serially diluting individual bacterial suspensions. High and low concentrations of *L. monocytogenes* on hotdogs were obtained by inoculating samples with 8 $\log_{10}$ CFU/mL and 4 $\log_{10}$ CFU/mL suspensions, respectively.

Hotdog manufacture. Hotdogs were manufactured at a USDA-inspected commercial hotdog processing facility owned by Kelley Foods, Elba AL. Mechanically deboned chicken (51%; 459 lbs containing 17% fat) meat and pork trim (49%; 441 lbs containing 26.4% fat) containing salt (1.3%) and nitrite (0.12%) at 2-4°C was ground once through 1 inch die plate grinder (Weiler and Co. Inc., WI) and then twice though

5/32 inch plate grinder (Robert Reiser and Co. Inc., MA). Ground meat was then mixed with bologna/frank seasoning (14 oz/25 lb, Blend 125, AC Legg, Inc., AL) in a spice mixer (Griffith Laboratories, IL). The spice mix comprised of sugar, dextrose, sodium erythroborate, monosodium glutamate, onion and garlic powder, sodium erythroborate, spice extractives and tricalcium phosphate. Seasoned meat was divided into 4 equal sections of 233 lbs and used for different smoke treatments. Liquid smoke (Zesti Smoke^{TM}, Kerry Ingredients and Flavours, TN) was added at 0, 2.5, 5 and 10% w/w to each section and mixed separately for 5 min in the spice mixer for uniform distribution. Ice was added to each section to compensate for the weight of liquid smoke and maintain the temperature below 4°C. Following mixing, the meat was emulsified through mince master (Griffith Laboratories, IL) and then stuffed into colorless striped cellulose casings (Viscofan USA Inc., Montgomery, AL) using a vacuum stuffer (Handmann Stuffer, Handmann Inc., IL). Hotdogs were linked using an automatic linker (Townsend Flax Linker NL 17, IA) to give 8 hotdogs/lb of green weight. Hotdogs were prepared in the order of 0, 2.5, 5 and 10% to prevent carryover of smoke from higher concentrations to lower. Stuffed hotdogs were cooked without external smoke application to final internal temperature of 73.89°C (165°F) and cooled over night at 4°C. Cooled hotdogs were then stripped off the casings using an automatic casing stripper and bulk packed separately according to treatments as 5 lb bags in corrugated boxes. The boxes were placed on ice in coolers (Igloo Quick & Cool, 100 Qt, Igloo, TX), transported to Auburn University Poultry Farm and maintained at 4°C until further repacking.

Packaging and Storage of hotdogs for shelf-life estimation. Hotdogs (n=6000) from each smoke treatment were divided into four sections (1500 hotdogs/section) of

which one section (1500 hotdogs) of each treatment was vacuum-packed for shelf-life determination. These hotdogs were randomly distributed into three trials and each trial packed as 4 hotdogs/bag x 3 bags/sampling x 3 replications x 4 treatments x 12 weeks; in nylon/PE vacuum bags (standard barrier, 20.3 x 25.4 cm, 3 mil; Prime Source Vacuum Pouches, Koch Supplies, Inc., MO). Vacuum-packed hotdogs were randomly distributed in a walk-in cooler (Thermo-Kool, Mid-South Industries, Inc., MS) maintained at 4°C.

Inoculation and packing of hotdogs for *Listeria*-growth study. All six *L. monocytogenes* suspensions (2 populations: high (8 log CFU/mL) and low (4 log CFU/mL) for each of 3 trials) prepared as mentioned in section "**Bacterial culture preparation**" were collected in sterile spray bottles of 1000 mL capacity and used for inoculating hotdogs. The remaining 3 sections of hotdogs (4500 hotdogs) from each treatment were separated into three trials and each trial packed as 4 hotdogs/pack x 3 bags/sampling x 3 replications x 12 weeks x 2 levels of inoculation. Prior to sealing, hotdogs in each bag were sprayed (1 mL) with either high or low concentrations of *L. monocytogenes*. Spray inoculated samples were hand massaged for 15 s for even distribution of the inocula. These bags were vacuum-packed and stored at 4°C in a separate walk-in cooler (Thermo-Kool, Mid-South Industries, Inc., MS) (Morey et al. 2010).

Sampling for shelf-life determination and *Listeria*-growth study. Vacuum-packed non-inoculated hotdogs (3 bags/sampling/trial/treatment) were obtained from the 4°C storage and 1 out of the 4 hotdogs from each bag was sampled for determining microbial shelf-life (later transferred to whirl-pak bag®), another one for color and texture (qualitative) analysis and remaining 2 for sensory evaluation. Hotdogs for sensory

and qualitative analyses from all bags of same trial were consolidated into zip-loc® bags giving 6 hotdogs/treatment/trial for sensory and 3 hotdogs/treatment/trial for qualitative analyses. All the hotdogs were stored at 4°C until further analyses except for qualitative analysis samples stored at room-temperature.

Inoculated hotdogs (3 bags/sampling/trial/treatment/inoculation level) stored at 4°C were randomly selected for studying the listeriostatic/cidal efficacy of liquid smoke. Samples (1 hotdog/bag) were aseptically collected in a sterile whirl-pak bag® (24 oz/710 mL 3 mil; Nasco, WI).

Microbiological analysis. Inoculated and non-inoculated samples in whirl-pack bags were rinsed with 25 mL of 0.1% peptone water (PW; Acumedia, MD) for 1 min. The rinsate from inoculated hotdogs was serially diluted and spread plated (0.1 mL) on Modified Oxford agar (MOX; Acumedia, MD) with *Listeria* supplement (Dalynn Biologicals, AB, Canada). Plates were incubated for 24h at 37°C, colonies exhibiting *Listeria*-like characteristics on MOX agar were counted and reported as $\log_{10}$ CFU/mL of rinsate.

Similarly, non-inoculated sample rinsate (0.1 mL) was spread-plated on plate count agar (PCA; Acumedia, MD), potato dextrose agar (PDA; Acumedia, MD), de Man Rogosa and Sharpe (MRS; Oxoid Ltd., UK) agar and violet red bile (VRB; Acumedia, MD) agar for the estimation of total aerobic plate count, yeast and molds, *Lactobacilli* and total *Coliforms* respectively. The PCA and VRB plates were incubated at 37°C for 48 h while PDA was incubated at room temperature for 5 days. MRS was incubated under anaerobic conditions in anaerobic chambers (7 Lts, Pack-Rectangular Jar, AnaeroPack™ System, Mitsubishi Gas Co. Inc., Japan) using AnaeroGen™ (Oxoid Ltd., UK) at 25°C

for 48 h. Colonies exhibiting typical characteristics on respective agars were counted and reported as $\log_{10}$ CFU/mL of rinsate.

Quality attributes. Non-inoculated hotdogs were used to determine quality attributes. Hotdogs (3 hotdogs/trial/treatment) collected in zip-lock bags were brought to room temperature and then used for texture profile analysis. Texture analysis was performed by cutting each hotdog to obtain 2 samples of 2.5 cm each and used for texture measurement (2 samples/hotdogs x 3 hotdogs/treatment x 3 trials). Texture profile analysis (TPA; TA.XTplus Texture Analyser, Texture Technologies, Scarsdale, NY) was conducted on prepared hotdog samples to determine the hardness, springiness, and chewiness of the samples. Each sample was subjected to two consecutive cycles with 25% compression with 5 s between each cycle using a 4 cm diameter probe moving at 1 mm/s. Hardness (gram force) is the measure of peak force required for first compression. Springiness (cm) is an indicator of ability of the product to "spring back" after compression while chewiness is a product of gumminess and springiness. These measurements were calculated using the software provided with the texture analyzer.

Sensory analysis. Non-inoculated hotdogs (6 hotdogs/treatment/trial) collected in zip-lock bags and stored at refrigerated temperature (4°C) were evaluated for consumer acceptability in two separate trials. Sensory analysis was conducted as two separate trials each week until week 12 wherein hotdog samples from trial 1 and 2 were served to an untrained panel in the morning and afternoon, respectively. During sensory evaluation, hotdogs from each treatment were coded and heated separately in water (65-70°C) for 10 min, cut into bite-sized pieces (ca. 2 cm) and placed in sampling cups with lids (Solo Cup Company, Highland Park, IL). Samples were kept in a warming oven (FlavorView C175-

C(1)N Heated Cabinet, Intermetro Industries Corp., Wilkes-Barre, PA) at ca. 75 °C until served to the panel. An untrained taste panel (30 panelists/ trial) comprising of employees and students at Auburn University evaluated hotdogs for appearance, flavor, texture, juiciness, and overall acceptability on an 8-point hedonic scale (Institute of Food Technologists, 1981). Warm, coded samples along with unsalted crackers, water and an evaluation form were provided to the panelists. The panelists were instructed to cleanse palate by taking a sip of water, eating a bite of cracker and sipping water before and after each sample. Samples were served one at a time and each panelist evaluated hotdog samples based on their degree of liking. Each attribute was scored on the same 8-point hedonic scale: (8) Like Extremely; (7) Like Very Much; (6) Like Moderately; (5) Like Slightly; (4) Dislike Slightly; (3) Dislike Moderately; (2) Dislike Very Much; and (1) Dislike Extremely.

Statistical analysis. Experiments were conducted in triplicate except for sensory analysis was done in duplicates and data was analyzed using SAS 9.1 software (SAS Institute, Cary, N.C.). Following analysis of variance, significant differences in the treatments were determined with Tukeys' LSD test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of liquid smoke on *L. monocytogenes*. Growth of *L. monocytogenes* was studied on hotdogs manufactured with 0, 2.5, 5 and 10% liquid smoke as an ingredient, stored under vacuum under refrigeration for 12 weeks (Figure 1a and b). Liquid smoke did not show any listericidal effect but suppressed ($p \leq 0.05$) the growth of *L. monocytogenes* inoculated at high and low levels on frankfurters throughout the storage period. Listeriostatic activity increased ($p \leq 0.05$) with increase in liquid smoke

concentrations with 10% showing the highest activity with no change ($p>0.05$) in *L. monocytogenes* counts throughout 12 weeks.

L. monocytogenes inoculated on hotdogs with no liquid smoke treatment (control) rapidly entered log phase and increased from 2.60 to 3.66 $\log_{10}$ CFU/mL in 7 days and ~8 logs in 12 weeks at 4°C under vacuum storage confirming its ability to rapidly adapt and grow under psychrotrophic and anaerobic conditions (Figure 1b). Similarly, Stohs (2009), Martin et al. (2009) and Bowers (2008) found that frankfurters without any antimicrobials inoculated with *L. monocytogenes* and stored under vacuum at 4°C reached ~10 logs in 12 weeks.

It is recommended to store RTE poultry and meat products like hotdogs at or below 4°C since low temperatures affects membrane permeability, reduces nutrient uptake, protein synthesis, and enzyme functionality, hence contributing to reduced growth (Graumann and Marahiel, 1999). On the other hand, *L. monocytogenes* exhibits growth on certain foods at temperatures as low as -0.4°C (ICMSF, 1996) by overcoming these factors through synthesis of cold-shock and acclimation proteins and other enzymes assisting in cell functions like uptake of solute, RNA helicases initiating gene translation at low temperatures and incorporating branched chain fatty acids in the cell membrane thus increasing membrane fluidity (Chan and Weidmann, 2008). Buchanan and Klawitter (1991) observed that *L. monocytogenes* cultured either aerobically or anaerobically at 37°C and then at 5°C rapidly adapts to the temperature change and exhibits comparable lag phase durations, exponential growth rates, generation times and maximum population densities. These alterations in cell physiology and functionality can

help *L. monocytogenes* survive the refrigerated temperatures at which the RTE foods are stored.

The growth of *L. monocytogenes* at 4°C was suppressed ($p \leq 0.05$) with the addition of liquid smoke at 2.5, 5 and 10% over a period of 12 weeks of storage (Figure 1a, b). Growth patterns indicated that although *L. monocytogenes* adapted to 4°C, addition of 2.5% liquid smoke suppressed growth ($p < 0.05$) and extended the log phase compared to 0% (control) to obtain a final cell density of 6.20 and 7.47 $\log_{10}$ CFU/mL of rinsate in low and high level inoculated hotdogs after 12 weeks. Similarly, growth of *L. monocytogenes* on hotdogs treated with 5% liquid smoke was further suppressed compared to 0 and 2.5% treatments ($p \leq 0.05$) and exhibited listericidal activity after week 6 and 3 at low and high levels of inoculums, respectively. On the other hand, addition of 10% smoke suppressed ($p \leq 0.05$) the growth of *L. monocytogenes* throughout the storage period among all the treatments reaching the final cell population of 2.28 and 5.94 logs CFU/mL of rinsate after 12 weeks for low and high inoculums respectively.

Liquid smoke has been used widely in the RTE products to reduce risks of *L. monocytogenes*. The antimicrobial compounds like the phenols, carbonyls and organic acids are condensed in water or other medium which are used as sprays or dips on products like frankfurters (Holley and Patel, 2005; Vitt et al. 2001). Various authors have studied the effectiveness of commercial liquid smoke preparations against *L. monocytogenes*. Murphy et al. (2005) demonstrated that liquid smoke (Select 23P) dispensed on franks effectively reduced *L. monocytogenes* inoculated on product surface by 3.2 $\log_{10}$ CFU/sq. cm in 4h and to less than 1 $\log_{10}$ CFU/ sq. cm at the end of 12 d storage at 4.4°C due to acidic pH (2.4) and phenolic compounds in the condensate.

Similarly, Gedela et al. (2007a) found that topical application of commercial liquid smoke preparation Zesti-B^{TM} on frankfurters and fully-cooked turkey chubs reduce ($p \leq 0.05$) *L. monocytogenes* by 2 logs within a week. Faith et al. (1992) found that CharSol Supreme^{TM} at 0.2 and 0.6% levels in wiener exudates inactivated *L. monocytogenes* within 1 to 5 days respectively and stated that the antimicrobial activity is due to presence of phenols. On the contrary, Sunen et al. (2001) demonstrated that higher phenol concentrations in commercial liquid smokes do not correlate with higher antimicrobial activity against *L. monocytogenes*. Faith et al (1992) reported that it is not the concentration but the kinds of phenol present in liquid smoke that affects it antimicrobial potential. They found that isoeugenol at less than 50 ppm concentration can inhibit ($p \leq 0.05$) *L. monocytogenes* as compared to other phenols like cresol, euginol and guaiacol. In addition to phenols, carbonyl compounds in liquid smoke can have antimicrobial effects. Carbonyls can sequester nutrients, inactivate or immobilize extracellular bacterial enzymes for metabolism and can also modify substrates leaving them unavailable for bacterial enzyme action (Milly, 2000). Along with phenols and carbonyls, organic acids are added to the liquid smoke imparting antimicrobial properties (Faith et al. 1992; Gedela et al. 2007a). Liquid smoke with low pH can be applied on the surface to give the desirable antimicrobial effect without affecting ($p > 0.05$) texture or color of the final product but cannot be incorporated in the product as it can lead to reduction in overall pH of the meat system and will affect meat emulsion, texture and quality of products like hotdogs. Significant research has been conducted on using liquid smoke as a spray or dip on ready-to-eat products while using it as an ingredient is a novel approach to apply liquid smoke. There are no published reports on the use of liquid

smoke as an ingredient against *L. monocytogenes*. Incorporation of Zesti Smoke™ as an ingredient exhibited listeriostatic rather than listericidal nature probably due to high pH compared to the ones used by Faith et al. (1992) and Gedela et al. (2007a,b). On the other hand, Zesti Smoke™ might consist of unique smoke components/compounds which are solely capable of suppressing the growth of *L. monocytogenes* without synergism with organic acids.

As compared to the published literature on conventional chemical antimicrobials like sodium lactates and diacetates, Zesti Smoke™ showed a very low antilisterial activity. Several researchers have used lactates either alone or in combination of diacetates as ingredients or post-lethality dip in meat products. Frankfurters formulated with 3% sodium lactate showed a listeriostatic effect until 70 days while the ones with 6% exhibit listericidal activity for 120 days at 4°C (Bedie et al. 2001). Similarly, listeriostatic effect of 3.3% sodium lactate as a sausage ingredient extended the lag phase by at least 2 weeks (Choi and Chin, 2003). The possible reason for the differences in the antimicrobial properties of lactates and diacetates as compared to liquid smoke are due to the chemical composition and the mode of action. Contrary to the effects of phenols and carbonyls in liquid smoke, lactates and diacetates reduce water activity and lower intracellular pH leading to impaired cell functions and growth (Shelef, 1994) rendering them more effective antimicrobials.

Although lactic acid and its salts are the most common and effective antimicrobial additives in RTE poultry and meat products; they can have significant effect on the organoleptic acceptability of the product (Carroll et al. 2007; Bowers, 2008). Moreover, these antimicrobials are synthetic and contradict the market trends of “clean labeling”.

Zesti Smoke™ was effective in suppressing the growth of *L. monocytogenes* when incorporated at 5 and 10% levels as hotdog ingredient. Addition of organic acids to lower the pH could possibly improve the efficacy of Zesti Smoke™.

Microbiological quality of hotdogs treated with liquid smoke. Non-inoculated hotdog samples were analyzed weekly for growth of total aerobic plate count, yeast and molds, *Lactobacilli* and total *Coliforms*. Results showed that bacterial and yeast and mold counts were 1 $\log_{10}$ CFU/mL or below for almost all the samples throughout the study irrespective of liquid smoke treatment. Liquid smoke exhibits antimicrobial activity against bacteria like *Yersinia entericolitica*, *Aeromonas hydrophila* and molds like *Penicillium, Aspergillus* and *Mucor* spp. on food products (Sunen et al. 2001; Wendorff and Wee, 1997). No clear trends between the control (0% smoke) and smoke treatments throughout the storage study indicate that the products were cooked thoroughly, handled and packed hygienically post-cooking and maintained under vacuum at 4°C to prevent the growth of any of the above bacteria.

Organoleptic evaluation of hotdogs. Non-inoculated hotdogs manufactured with liquid smoke at 0, 2.5, 5 and 10% levels stored under vacuum at 4°C were evaluated for their organoleptic acceptance for 12 weeks. Pre-warmed samples were evaluated for their appearance, texture, juiciness, flavor and overall acceptability.

Appearance. Appearance and color of a product are the first attributes that consumers perceive creating a halo effect leading to modifications in flavor recognition, overall sensory experience and acceptability of the product (Hutchings, 1977; Maga, 1974; Kostyla and Clydesdale, 1978). Product appearance can be altered by addition, deletion or substitution of ingredients especially those imparting color among many other

factors. Additives like liquid smoke are available in wide color ranges from dark to pale depending on the product manufacturer and specifications (Gedela et al. 2007b) and can impart color and change product appearance. Incorporation of liquid smoke in meat emulsion favors the development of a stable desired cured color (Sink and Hsu, 1979). Zesti Smoke^{TM} has a pale yellow color and its addition in increasing percentage of 0, 2.5, 5 and 10% were tested on the appearance of the hotdogs. Contrary to the findings of Sink and Hsu (1979), untrained consumer panelists indicated same degree of likeness ($p>0.05$) for appearance of hotdogs (Table 1) made with various concentrations of smoke indicating that Zesti Smoke^{TM}. Panelists rated the appearance as "liked moderately" at the beginning of the study which lowered to "like slightly" by the end of 12 weeks (Table 1). These observations indicate that addition of liquid smoke did not ($p>0.05$) alter the consumers response to the likeness of appearance of hotdogs.

Texture. Texture of food products is dependent on the composition of the food and is an important attribute of the product. Softer texture of poultry hotdogs is due to the nature of muscle proteins, less collagen, more unsaturated fatty acids as compared to beef hotdogs that have firmer texture. Several attempts have been made to use different additives like carrageenan, cellulose gums, oats, and grape seed extract to improve the texture of frankfurters or use them as fat replacers (Hughes et al. 1997; Barbut and Mittal, 1996; Özvural and Vural, 2011). Liquid smoke consists of carbonyls that can interact with muscle foods, affect their textural properties (Martinez et al 2004) and ultimately consumer acceptance. It should be noted that hotdogs made in this study did not have any additional binders, extenders or other additives to improve the texture of the product.

Although most studies use liquid smoke on surface of the product, it is expected that such application might not change the textural properties of the product. On the other hand, it has been observed that incorporation of liquid smoke in meat emulsion renders it less desirable or acceptable (Sink and Hsu, 1979). Addition of liquid smoke at 2.5, 5 and 10% in hotdogs did not affect ($p>0.05$) the texture scores of hotdogs as compared to 0% treatment (Table 1). Hotdogs made with 10% liquid smoke were found "chewy" and "slightly dry" by the panelists. No significant differences ($p>0.05$) in texture of 2.5 and 5% hotdogs indicated that there was no perceivable change in texture as compared to the control hotdogs (Table 1). Although, taste-panelists rated the texture above "like moderately" in the beginning of the study, scores dropped to "like slightly" by the end of 12 weeks. Hence it can be concluded that liquid smoke (Zesti Smoke^{TM}) does not affect the texture of the hotdogs.

Juiciness. Juiciness is a sensory measurement of moisture-holding capacity of the product. Although, chemical compounds in liquid smoke like the phenols and carbonyls bind to the water in the product making it firmer and less juicier or drier (Sink and Hsu, 1979), panelists did not perceive any differences ($p>0.05$) in the juiciness values of hotdogs containing 2.5, 5 and 10% liquid smoke compared to the control samples (Table 1).

Juiciness is also affected by the fat content of hotdogs (Barbut and Mittal, 1996). The formulation used in this study consisted of mechanically deboned chicken and pork trim with fat. Pork has more saturated fatty acids than poultry, which improves texture and provides a juicier mouthfeel to the product (Keeton, 1983; Sams and Diez, 1991).

The effect of pork fat on juiciness differences in this study is very unlikely since the same uniformly ground chicken and pork mixture was used for all the treatments.

Moreover, juiciness can also be affected by the pH of the additives. Bowers (2008) found that addition of sodium lactate and diacetate in combination reduced the pH of the meat emulsion giving a drier mouthfeel to the product. This effect of pH might not have played a role in juiciness values since the pH of Zesti Smoke^{TM} was near neutral and its addition did not ($p>0.05$) affect the pH of the meat emulsion.

Juiciness values decreased ($p\leq0.05$) as storage progressed from week 1 to week 12. Taste-test panelists rated juiciness slightly below "liked moderately" on the sensory scale (Table 1). Although, some panelists commented that the products were drier, probably due to moisture loss during storage, these results indicate that panelists equally liked ($p>0.05$) the juiciness of hotdogs irrespective of liquid smoke.

Flavor. Liquid smoke preparations contain aldehydes, ketones, phenols, caboxylic acids, hydrocarbons among the mixture of various chemical components which can impart a smoky flavor to the product (Messina et al. 1988; Guillen et al., 1995). Addition of Zesti Smoke^{TM} at 2.5 and 5 % did not influence ($p>0.05$) the flavor profile of the product compared to the control (Table 1). Incorporation of 10% liquid smoke received lower scores ($p\leq0.05$) on week 2, 3, 4, 5 and 7 (Table 1). Taste panelists perceived the 10% treatment as "extremely smoky" and rated the product "liked slightly". Although hotdogs incorporated with 10% liquid smoke were prepared from the same mixture of meat and seasonings, the panelist commented that they were "more salty" than the others indicating that smoke may enhance salty taste of the product.

Chicken has a very low flavor profile as compared to stronger flavors in turkey or other meats and can readily take up added flavors. Sensory scores ($p>0.05$) indicated that there was no significant difference in the acceptability levels, in terms of flavor, of hotdogs having liquid smoke. This observation can be either due to low smoky flavor of Zesti Smoke^{TM} , or the flavor is masked by strong frankfurter seasonings (Carroll et al. 2007). It is also possible that there was a change in the flavor due to addition of liquid smoke but panelists liked and ranked the products at same level. Lower scores for 10% samples indicate that Zesti Smoke^{TM} can be added up to 5% without negatively impacting flavor profile of hotdogs.

Overall. Overall acceptability sensory scores (Table 1) give an estimate of complete sensory experience of a consumer and the desire to buy a particular product. Sensory evaluations panelists scored the hotdogs incorporated with 10% liquid smoke samples lower ($p\leq0.05$) than the other two smoke treatments, which were not different ($p>0.05$) than the control (no-smoke added; 0% samples). This trend might be due to the alteration in the flavor of the product as a result of 10% smoke. Taste panelist rated the 0, 2.5 and 5% products as “liked moderately” compared to 10% which was rated above “liked slightly” (Table 1) indicating that an acceptable product could be made without additives to enhance the texture, juiciness and flavor other than hotdog seasoning.

Overall acceptability scores decreased overtime with week 12 samples having lower scores than week 1 (Table 1). Organoleptic evaluations indicate that Zesti Smoke^{TM} can be added without affecting ($p>0.05$) the sensory qualities of the product.

Texture profile analysis. Hotdog samples were subjected to objective analysis to determine the differences in hardness, springiness and chewiness due to addition of liquid smoke as an ingredient (Table 2).

Hardness of the chicken/pork hotdogs manufactured with various concentrations of smoke ranged from approximately 1300 to 2300 g force which is lower than hotdogs made from other species (Yang et al. 2001; Bratcher et al., 2011). These differences are due to difference in muscle proteins, use of binders and fillers contributing to increased texture of the product. Although hotdogs were made with chicken and pork without any additives to improve texture, hardness scores and organoleptic evaluations indicated that consumer acceptable hotdogs can be made without any of above additives.

Hardness and chewiness increased ($p \leq 0.05$) with increase in the smoke concentrations from 0 to 10% (Table 2) indicating that the chemical components in smoke interacted with meat emulsion affecting the firmness of the hotdogs (Martinez et al. 2004). Phenolic compounds in liquid smoke interact with water to increase tenderness and lower the shear-force values in the hotdogs (Sink and Hsu, 1979). On the other hand, carbonyl compounds interact with proteins to increase the firmness of the product (Guillen et al. 1995; Martinez et al. 2004). However, springiness of hotdogs was not affected ($p > 0.05$) due to addition of smoke in the meat emulsion (Table 2).

Comparison of instrumental analysis of texture to the organoleptic evaluations indicated that hardness of 1500 g (force) and chewiness of 1400 can give lower texture scores to the hotdogs made with liquid smoke (Table 1 and 2). Untrained panelists commented that the hotdogs with 10% liquid smoke were “chewier” than the others

which can be correlated with the chewiness data wherein 10% samples had higher ($p \leq 0.05$) chewiness readings than the other samples.

Based on the results, it can be concluded that liquid smoke can be added as an ingredient in hotdogs to replace antimicrobials like lactates and diacetates. In addition, it can be effectively used to replace additives like binders, fillers and emulsifiers in chicken/pork hotdogs to give a consumer acceptable product. Hotdogs manufactured with liquid smoke showed promising applications as it was able to prevent the growth of *L. monocytogenes* at 4°C under vacuum packaging and without affecting ($p > 0.05$) organoleptic properties. Liquid smoke can be used as "Alternative 1" to control *L. monocytogenes* in RTE products. Moreover, addition of liquid smoke can cater to the current consumer demand for an "all natural" and "clean labeled" product.

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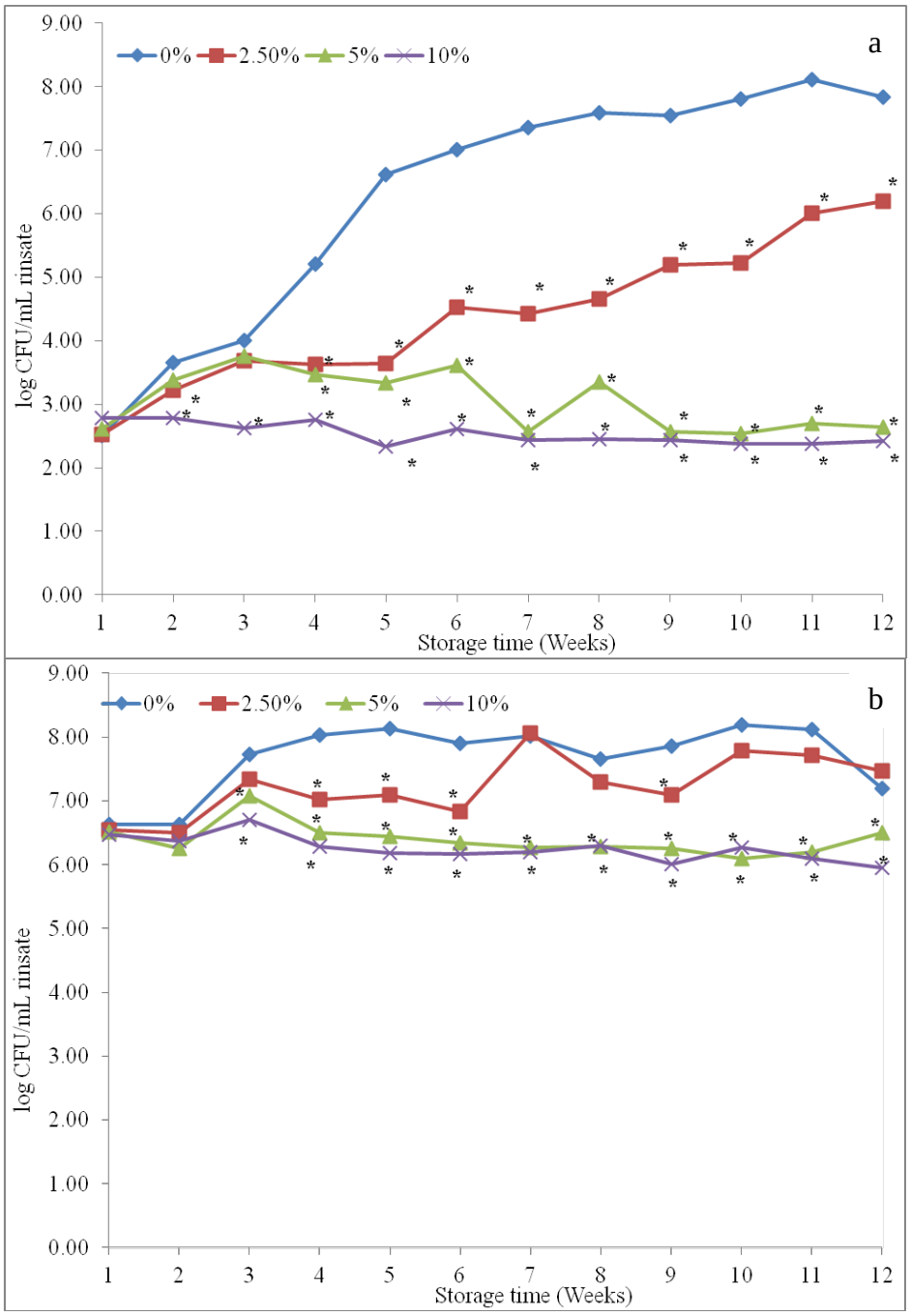

Figure 1. Growth patterns of *L. monocytogenes* inoculated at (a) high and (b) low levels on vacuum packaged hotdogs stored at 4°C for 12 weeks

* indicates difference ($p \leq 0.05$) in *L. monocytogenes* counts compared to control within each week.

Table 1. Organoleptic evaluation scores (± standard deviation) of hotdogs manufactured with 0, 2.5, 5 and 10% liquid smoke as an ingredient for 12 weeks*

Storage Period	Smoke Concentration (%)	Appearance	Texture	Juiciness	Flavor	Overall
Week 1	0	6.47±0.91[a]	6.10±0.99[a]	6.40±0.96[a]	6.10±1.12[a]	6.10±1.00[a]
	2.5	6.45±0.85[a]	6.38±0.85[a]	6.47±0.81[a]	6.10±1.10[a]	6.10±0.95[a]
	5	6.50±0.85[a]	6.10±1.07[a]	6.42±0.91[a]	6.15±0.90[a]	6.13±0.91[a]
	10	6.47±0.87[a]	6.00±1.25[a]	6.38±0.88[a]	5.65±1.27[a]	5.77±1.11[a]
Week 2	0	6.52±0.82[a]	6.52±0.86[a]	6.48±1.00[a]	6.30±0.90[a]	6.37±0.91[a]
	2.5	6.72±0.84[a]	6.32±1.03[a]	6.42±1.04[a]	6.26±1.32[a]	6.35±1.15[a]
	5	6.52±1.00[a]	6.25±1.11[a]	6.33±1.08[a]	6.04±1.15[ba]	6.25±1.11[a]
	10	6.64±0.94[a]	6.23±1.19[a]	6.30±1.11[a]	5.64±1.49[b]	5.86±1.35[a]
Week 3	0	6.30±0.93[a]	6.18±1.13[a]	6.42±0.96[a]	6.27±1.07[ba]	6.30±1.01[a]
	2.5	6.43±0.93[a]	6.32±1.02[a]	6.32±0.97[a]	6.15±1.15[ba]	6.30±0.98[a]
	5	6.58±0.81[a]	6.37±1.02[a]	6.15±1.01[a]	6.29±0.98[a]	6.37±0.93[a]
	10	6.48±0.77[a]	6.10±1.10[a]	6.00±1.19[a]	5.75±1.32[b]	5.72±1.22[b]
Week 4	0	6.26±0.82[a]	6.17±1.12[a]	6.39±0.89[a]	6.24±1.25[ba]	6.29±1.18[a]
	2.5	6.15±1.15[a]	6.07±1.19[a]	6.17±0.99[ba]	6.28±1.33[a]	6.20±1.16[a]
	5	6.23±1.05[a]	6.20±1.05[a]	6.12±1.11[ba]	6.15±1.19[ba]	6.20±1.13[a]
	10	6.15±0.97[a]	6.00±1.24[a]	5.70±1.27[b]	5.63±1.45[b]	5.78±1.21[a]
Week 5	0	6.52±0.91[a]	6.17±1.09[a]	6.33±1.26[a]	6.30±1.14[a]	6.27±1.04[ba]
	2.5	6.63±0.88[a]	6.47±0.89[a]	6.32±1.05[a]	6.55±1.00[a]	6.52±0.85[a]
	5	6.60±0.94[a]	6.39±1.08[a]	6.17±1.05[a]	6.25±0.92[a]	6.22±1.02[a]
	10	6.25±1.25[a]	5.94±1.56[b]	5.84±1.34[a]	5.71±1.25[b]	5.80±1.36[b]
Week 6	0	6.46±1.11[a]	6.26±1.02[a]	6.25±0.99[a]	6.19±0.98[a]	6.22±1.08[a]
	2.5	6.71±0.92[a]	6.16±1.12[a]	6.36±1.22[a]	6.22±1.07[a]	6.24±1.04[a]
	5	6.60±0.76[a]	6.27±1.27[ba]	6.20±1.19[a]	5.95±1.11[a]	6.00±1.31[ba]
	10	6.66±0.85[a]	6.06±1.26[b]	5.92±1.13[a]	5.66±1.04[a]	5.68±1.23[b]

Table 1. continued…

Storage Period	Smoke Concentration (%)	Appearance	Texture	Juiciness	Flavor	Overall
Week 7	0	6.63±0.91[a]	6.35±1.26[a]	6.35±0.85[a]	6.37±1.08[a]	6.32±0.99[a]
	2.5	6.56±1.05[a]	6.22±1.30[a]	6.45±1.24[a]	6.35±1.27[a]	6.32±1.27[a]
	5	6.64±0.83[a]	6.35±1.03[a]	6.30±1.09[a]	6.23±1.21[ba]	6.18±1.17[ba]
	10	6.45±1.01[a]	6.00±1.11[a]	6.00±0.95[a]	5.71±1.34[b]	5.69±1.34[b]
Week 8	0	6.12±1.25[a]	5.83±1.45[a]	5.78±1.36[a]	5.67±1.25[a]	5.67±1.52[a]
	2.5	6.16±1.40[a]	6.03±1.59[a]	6.00±1.55[a]	5.90±1.25[a]	5.91±1.51[a]
	5	6.17±1.19[a]	5.78±1.41[a]	5.80±1.49[a]	5.66±1.21[a]	5.65±1.51[a]
	10	6.19±1.18[a]	5.77±1.36[a]	5.78±1.28[a]	5.63±1.25[a]	5.56±1.33[a]
Week 9	0	6.11±1.27[a]	5.89±1.63[a]	6.11±1.27[a]	5.56±1.33[a]	5.60±1.63[ba]
	2.5	6.13±1.34[a]	5.98±1.43[a]	6.02±1.34[a]	5.63±1.24[a]	5.80±1.27[ba]
	5	6.11±1.30[a]	5.93±1.42[a]	6.05±1.36[a]	5.80±1.24[a]	5.88±1.42[a]
	10	5.89±1.45[a]	5.40±1.82[a]	5.75±1.63[a]	5.11±1.58[a]	5.06±1.80[b]
Week 10	0	6.25±1.17[a]	6.18±1.19[a]	6.32±0.98[a]	5.93±0.92[a]	6.05±1.15[a]
	2.5	6.25±0.98[a]	6.27±1.21[a]	6.40±0.91[a]	6.16±0.96[a]	6.16±0.98[a]
	5	6.22±1.08[a]	6.00±1.58[a]	6.24±1.45[a]	5.78±0.96[a]	5.89±1.39[a]
	10	5.91±1.36[a]	5.69±1.46[a]	6.09±1.41[a]	5.47±0.88[a]	5.71±1.38[a]
Week 11	0	6.23±1.30[a]	6.02±1.46[a]	6.22±1.31[a]	5.86±1.13[a]	6.06±1.33[a]
	2.5	6.05±1.50[a]	5.96±1.50[a]	6.26±1.36[a]	5.84±1.31[a]	5.75±1.54[ba]
	5	5.99±1.50[a]	5.88±1.48[a]	6.04±1.22[a]	5.89±1.39[a]	6.00±1.40[a]
	10	5.90±1.34[a]	5.46±1.53[a]	5.76±1.42[a]	5.25±1.15[a]	5.24±1.48[b]
Week 12	0	5.95±1.51[a]	5.83±1.52[a]	6.02±1.47[a]	5.81±1.17[a]	5.85±1.45[a]
	2.5	5.92±1.42[a]	6.05±1.61[a]	6.02±1.30[a]	5.73±1.29[a]	5.86±1.50[a]
	5	6.04±1.51[a]	5.83±1.68[a]	5.92±1.42[a]	5.67±1.31[a]	5.70±1.56[a]
	10	5.90±1.44[a]	5.72±1.53[a]	5.92±1.54[a]	5.23±1.08[a]	5.40±1.49[a]

* Different superscripts indicate differences ($p \leq 0.05$) for a particular attribute within each week.

Table 1 continued…

8-point hedonic scale: (8) Like Extremely; (7) Like Very Much; (6) Like Moderately; (5) Like Slightly; (4) Dislike Slightly; (3) Dislike Moderately; (2) Dislike Very Much; and (1) Dislike Extremely.

Table 2. Effect of liquid smoke added as an ingredient on the texture profile analysis (± standard deviation) of hotdogs for 12 weeks

Storage Period	Smoke Concentration (%)	Hardness (g Force)	Springiness (cm)	Chewiness
Week 1	0	1460.42±209.40^{b}	0.99±0.01^{a}	1296.73±188.68^{c}
	2.5	1507.53±169.39^{b}	1.00±0.15^{a}	1368.24±152.50^{bc}
	5	1747.87±117.49^{a}	1.05±0.22^{a}	1550.74±155.72^{ba}
	10	1910.66±364.96^{a}	0.98±0.03^{a}	1686.22±323.67^{a}
Week 2	0	1413.49±187.55^{c}	1.00±0.01^{a}	1253.40±190.09^{c}
	2.5	1548.94±161.04^{c}	1.04±0.19^{a}	1465.10±294.62^{c}
	5	1894.38±127.49^{b}	0.99±0.03^{a}	1702.56±127.76^{b}
	10	2103.79±267.54^{a}	0.99±0.04^{a}	1886.45±257.28^{a}
Week 3	0	1462.64±162.67^{b}	1.00±0.03^{a}	1304.22±157.14^{c}
	2.5	1589.79±207.04^{b}	1.00±0.02^{a}	1424.53±174.72^{bc}
	5	1618.34±170.18^{b}	1.01±0.07^{a}	1467.65±128.67^{b}
	10	2048.87±226.27^{a}	1.00±0.02^{a}	1835.10±206.24^{a}
Week 4	0	1462.64±162.67^{b}	1.00±0.03^{a}	1313.10±157.04^{c}
	2.5	1589.79±207.04^{b}	1.00±0.02^{a}	1424.53±174.72^{bc}
	5	1633.59±166.09^{b}	1.00±0.06^{a}	1480.36±124.81^{b}
	10	2091.46±225.01^{a}	1.00±0.02^{a}	1860.26±234.45^{a}
Week 5	0	1435.65±154.00^{c}	0.99±0.03^{b}	1262.69±142.24^{c}
	2.5	1606.65±202.92^{b}	1.00±0.00^{ab}	1440.53±159.23^{bc}
	5	1611.03±216.07^{b}	1.10±0.20^{a}	1559.64±297.26^{ba}
	10	1905.09±178.14^{a}	1.00±0.02^{b}	1732.02±181.26^{a}
Week 6	0	1599.94±233.13^{b}	1.07±0.19^{a}	1431.21±232.85^{c}
	2.5	1627.86±162.09^{b}	1.00±0.01^{a}	1466.24±108.40^{bc}
	5	1731.32±169.33^{b}	1.01±0.04^{a}	1589.51±159.40^{b}
	10	2013.94±166.57^{a}	0.99±0.02^{a}	1775.88±149.36^{a}

Table 2 continued…

Week 7	0	1454.12±104.80^{b}	1.00±0.01^{a}	1412.67±291.23^{ab}
	2.5	1457.23±137.92^{b}	1.00±0.00^{a}	1414.24±261.25^{ab}
	5	1510.27±207.13^{b}	0.99±0.03^{a}	1366.13±202.03^{b}
	10	1810.35±140.53^{a}	1.00±0.06^{a}	1641.97±212.00^{a}
Week 8	0	1431.36±177.03^{c}	1.02±0.12^{a}	1277.01±175.58^{c}
	2.5	1550.82±160.14^{b}	1.14±0.23^{a}	1575.95±263.21^{b}
	5	1624.37±109.10^{b}	1.07±0.22^{a}	1487.98±129.42^{bc}
	10	1920.90±162.05^{a}	1.12±0.32^{a}	1821.41±311.48^{a}
Week 9	0	1365.73±137.23^{c}	1.04±0.10^{a}	1357.86±165.13^{c}
	2.5	1531.40±184.57^{bc}	1.06±0.21^{a}	1373.50±157.00^{bc}
	5	1633.64±196.31^{b}	1.00±0.07^{a}	1594.95±362.61^{ba}
	10	2013.07±234.91^{a}	1.03±0.11^{a}	1816.02±193.69^{a}
Week 10	0	1631.71±177.69^{b}	1.00±0.07^{a}	1461.147±208.68^{ba}
	2.5	1529.88±198.28^{b}	1.00±0.02^{a}	1356.62±174.37^{b}
	5	1602.90±204.59^{b}	0.99±0.03^{a}	1417.30±175.64^{b}
	10	1873.44±254.62^{a}	1.00±0.02^{a}	1652.328±224.96^{a}
Week 11	0	1667.29±99.91^{c}	1.00±0.03^{a}	1483.29±94.66^{b}
	2.5	1815.76±128.14^{b}	1.00±0.02^{a}	1632.47±122.43^{b}
	5	1876.05±155.67^{b}	0.99±0.02^{a}	1817.32±673.57^{ba}
	10	2306.19±137.15^{a}	1.02±0.06^{a}	2109.62±187.80^{a}
Week 12	0	1603.44±116.47^{b}	1.02±0.03^{a}	1458.09±110.32^{ba}
	2.5	1577.97±198.49^{b}	1.01±0.03^{a}	1431.48±198.57^{b}
	5	1668.60±283.56^{ba}	0.99±0.04^{a}	1487.27±264.25^{ba}
	10	1867.23±258.60^{a}	0.99±0.02^{a}	1651.32±234.75^{a}

* Different superscripts indicate differences ($p \leq 0.05$) for a particular attribute within each week.