

APPLICATION OF LIQUID SMOKE ALONE AND  
IN COMBINATION WITH PRE-AND POST-  
PACKAGE PASTEURIZATION AGAINST  
*LISTERIA MONOCYTOGENES* ON  
READY-TO-EAT MEATS

By

SARITHA GEDELA

Bachelor of Science

Acharya N. G. Ranga Agricultural University

Andhra Pradesh, India

2000

Submitted to the Faculty of the  
Graduate College of the  
Oklahoma State University  
in partial fulfillment of the  
requirements for  
the Degree of  
MASTER OF SCIENCE  
December, 2005

APPLICATION OF LIQUID SMOKE ALONE AND  
IN COMBINATION WITH PRE-AND POST-  
PACKAGE PASTEURIZATION AGAINST  
*LISTERIA MONOCYTOGENES* ON  
READY-TO-EAT MEATS

Thesis Approved:

Dr. Peter Muriana

---

Thesis Advisor

---

Dr. William McGlynn

---

Dr. Christina A. Mireles DeWitt

---

Dr. A. Gordon Emslie

Dean of the Graduate College

## ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my advisor Dr Peter Muriana for his intelligent supervision, guidance, help and inspiration during this endeavor. With his supervision and advice, I could successfully finish my research. My sincere appreciation extends to my other committee members Dr. William McGlynn and Dr. Christina A. Mireles DeWitt, whose guidance, assistance, encouragement are also invaluable.

I am thankful to Dr. Stanley Gilliland, graduate coordinator for the graduate study in Food Science program, and I would like to thank the Department of Animal Science for supporting during the course of my study. I also thank Mastertaste Inc. and Bar-S Foods Inc. for providing product, technical, and/or financial assistance during the course of this project.

I am also thankful to my parents, my friends and my husband Hari whose love, encouragement, understanding and support made me to accomplish my career goal.

## TABLE OF CONTENTS

CHAPTER	PAGE
I. REVIEW OF LITERATURE	
1.1. <i>Listeria monocytogenes</i> .....	1
1.1.1. Organism characteristics .....	2
1.1.2. Listeriosis .....	3
1.1.3. Outbreaks and spread of listeriosis .....	4
1.2. <i>Listeria</i> significance in RTE Foods .....	9
1.2.1. Properties of <i>Listeria monocytogenes</i> in relation to food and food processing .....	9
1.2.2. Prevalence of <i>Listeria monocytogenes</i> in raw materials.....	10
1.3. Factors affecting growth and survival of <i>Listeria monocytogenes</i> .....	12
1.3.1 Temperature.....	12
1.3.2. pH.....	14
1.3.3. Water activity.....	15
1.3.4. Atmosphere.....	16
1.4. Government regulations.....	16
1.5. Effectiveness of inhibitory compounds in inhibiting the .....	19
growth of <i>Listeria monocytogenes</i>	
1.5.1. Chemical preservatives .....	19
1.5.2. Sodium lactate.....	22
1.5.3. Sodium diacetate.....	24
1.5.4. Sodium acetate .....	26
1.5.5. Plant essential oils.....	26
1.6. Smoke flavorings/extracts.....	28
1.6.1. Traditional smoking .....	28
1.6.2. Commercial smoking.....	29
1.6.2.1. Liquid smoke/purpose of smoking .....	30
1.6.2.2. Composition of smoking .....	31
1.6.2.3. Production of smoking.....	32
1.6.2.4. Inhibitory nature of liquid smoke.....	33
1.6.2.5. Advantages and disadvantages of liquid smoke.....	34
1.7. References.....	36
II. Effect of liquid smoke extracts on the shelflife of frankfurters inoculated with <i>Listeria monocytogenes</i>	
2.1. Abstract.....	48
2.2. Introduction .....	50
2.3. Materials and Methods.....	53

2.4. Results and Discussion .....	57
2.5. References .....	62
2.6. Figures .....	66
III. Effect of liquid smoke extracts in combination with heating on the shelf life of RTE meats inoculated with <i>Listeria monocytogenes</i>	
3.1. Abstract .....	70
3.2. Introduction .....	71
3.3. Materials and Methods .....	74
3.4. Results and Discussion .....	80
3.5. References .....	84
3.6. Figures .....	86
APPENDIX	
EFFECT OF MIONIX RTE-01 AGAINST <i>LISTERIA MONOCYTOGENES</i> ON READY-TO-EAT MEATS .....	92

## LIST OF TABLES

TABLE	PAGE
1. Clinical syndromes associated with <i>L. monocytogenes</i> .....	4
2. Outbreaks of listeriosis in the United States (1970-2002) with known food vehicles.....	8
3. A comparative ranking of types of food group vehicles by number of outbreaks and number of cases in the Unites States.....	9
4. USDA recall classification.....	19

## LIST OF FIGURES

### CHAPTER II

FIGURE	PAGE
1. The flow chart of different dipping times as well as the spray against the growth of <i>L. monocytogenes</i> .....	
2. Spray applicators used in this study.....	66
3. Inoculation of five retail brands of hotdogs.....	67
4. Retail hotdogs dipped and sprayed with liquid smoke extract.....	68
5. Treatment of hotdogs with liquid smoke by inoculation with three different levels of inoculum.....	69

### CHAPTER III

1. Diagrammatic representation of evaluation of 2 liquid smoke extracts for anti- <i>Listerial</i> activity on hotdogs.....	
2. Evaluation of 2 liquid smoke extracts for anti- <i>Listerial</i> activity on hotdogs.....	87
3. Liquid smoke (Zesti-B™) in combination with prepackage pasteurization of deli turkey breasts manufactured without lactate/diacetate.....	88
4. Liquid smoke (Zesti-B™) in combination with post-package pasteurization of deli turkey breast.....	89
5. Effect of Zesti-B™ (brown) vs. AM-3™ (clear) liquid smoke in combination with heat against <i>Listeria monocytogenes</i> .....	90

## CHAPTER 1

### LITERATURE REVIEW

#### 1.1. *Listeria monocytogenes*

*Listeria monocytogenes* is a major foodborne pathogen. *L. monocytogenes* generally enters the host during the consumption of contaminated food. It has been the cause of numerous sporadic illnesses and associated with high mortality rates in large outbreaks (Messina et al., 1988). Historically, Murray et al. (1926) were credited with the discovery of a small Gram-positive bacillus from the blood of infected rabbits in 1924. The word 'monocytum' refers to a blood cell 'monocyte' and monocytogenes means monocyte-producing. Since it produced a typical monocytosis during illness in the diseased animal, the organism was designated as *Bacterium monocytogenes*. Pirie (1940) isolated an organism from a gerbille (an African jumping mouse) that was very similar to the bacterium described by Murray et al. (1926) and named it *Listerella hepatolytica* in honor of Lord Lister, an English surgeon and the discoverer of antisepsis (Doyle, 1989). In 1939, it was discovered that the generic name *Listerella* had already been used for a group of slime molds in 1906. The proposed name was changed by Pirie (1940) from *Listerella monocytogenes* to *Listeria monocytogenes*, and approved later in 1954 by the



Judicial Commission on Bacteriological Nomenclature and Taxonomy (Ryser and Marth, 1991).

During the 1960's most of the listeriosis research in the United States was done on animals by M. L. Gray and his associates (Doyle, 1989). By 1985, eight species of *Listeria* were recognized in the Approved Lists of Bacterial Names. They are *L. monocytogenes*, *L. seeligeri*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. denitrificans*, *L. murrayi*, and *L. grayi* (Ryser and Marth, 1991). Although we can find these eight species of *Listeria* in the ninth edition of Bergey's Manual of Systematic Bacteriology, *L. grayi* and *L. murrayi* were considered for reclassification and placed in a newly created genus *Murraya*. *L. denitrificans* has been reclassified as *Jonesia denitrificans*. Studies using DNA/DNA hybridization technologies have shown that the degree of relatedness between *L. denitrificans* and other *Listeria* spp. was low and used as justification for the change (Ryser and Marth, 1991).

### **1. 1. 1. Organism characteristics**

*L. monocytogenes* can be found in soil, silage, plants, and water and can be isolated from numerous species of domestic and wild animals. It is characterized as small, short diphtheroid-like coccoid rods, 0.4-0.5 µm in diameter and 0.5-2.0 µm in length with rounded ends. It is a Gram-positive, non-sporeforming, non-acidfast, and pleomorphic bacterium. This organism is motile by peritrichous flagella when grown at 20-25°C. Only a few flagella can be found at 37°C. The characteristic of motility has also been useful in identifying the

organism (Doyle, 1989). *L. monocytogenes* is a facultative intracellular anaerobe, catalase-positive, oxidase-negative, and is beta-hemolytic. *L. monocytogenes* does not require CO<sub>2</sub> for growth, but the growth is enhanced with 5-10% CO<sub>2</sub>. This organism can grow well on media in the pH range of 5.0 to 9.0. After 24 hrs of incubation at 37°C on solid medium such as nutrient agar, colonies are characterized as round, translucent, slightly raised with a finely textured surface, watery in consistency, dew-drop-like and bluish when viewed under normal illumination (Ryser and Marth, 1991). *L. monocytogenes* is a psychotropic organism with an optimum growth temperature of 30-37°C, but has the ability to grow at a wide range of temperatures from 1-45°C (Gray, 1960). *L. monocytogenes* is known to survive under various conditions of refrigeration, freezing, heating, and drying, which provides difficulties for the food industry (CFSAN, 2003).

### **1. 1. 2. Listeriosis**

The consumption of either food or water contaminated with pathogenic microorganisms, microbial toxins, chemicals, or naturally occurring plant or animal toxins may result in a foodborne disease syndrome. Listeriosis is the common name given to the general group of disorders caused by the bacterium *L. monocytogenes*. Even though the concept of listeriosis as a foodborne illness was first suggested by Murray et al. (1926), the ingestion and penetration of *L. monocytogenes* in the gastrointestinal tract is one of the means by which listeriosis can occur (Doyle, 1989). People with underlying illnesses or the elderly

with weakened immune systems are more prone to listeriosis, which is a life-threatening foodborne disease. Immuno-suppressed individuals, pregnant women, fetuses and neonates are most susceptible to *Listeria* infection (CDC, 2002). Human listeriosis is characterized by high mortality rates, with clinical features that include mild influenza-like symptoms, encephalitis, meningitis, fatal bacteremia, puerperal sepsis, and a flu-like illness during pregnancy (Schlech, 1996). It can also cause miscarriages, stillbirths, preterm labor by cross-placenta and utero-fetal infection (Mclauchlin, 1996). The clinical symptoms associated with *L. monocytogenes* were listed in Table 1. The initial symptoms are often fever, muscle aches, and sometimes gastrointestinal symptoms such as nausea or diarrhea. In addition to oral infection, there is evidence that *L. monocytogenes* may infect humans and animals by ocular, cutaneous, respiratory, or urogenital routes (Seeliger and Jones, 1993). However, the minimum infectious dose of *L. monocytogenes* is currently unknown (NACMCF, 2003).

Table1. Clinical syndromes associated with *L. monocytogenes* (Schlech, 1996).

Meningitis	Osteomyelitis
Meningoencephalitis	Endocarditis
Brain abscess	Hepatitis
Rhombencephalitis	Liver abscess
Bacteremia	Cholecystitis
Endocarditis	Diarrhea (with foodborne outbreaks)
Spontaneous bacterial Peritonitis	Cutaneous infections (In animal workers)
Pneumonia	Endophthalmitis
Septic arthritis	

### 1. 1. 3. Outbreaks and spread of listeriosis

The Centers for Disease Control & Prevention (CDC, 2000) estimated that each year 76 million people get sick, more than 300,000 are hospitalized, and 5,000 die as a result of foodborne illness. Along with the health impact, foodborne illnesses have an economic impact estimated at 5 billion U.S. dollars (Altekruse et al., 1997).

Although foodborne listeriosis is rare, the associated mortality rate is higher among those that are most at-risk. The CDC (2003) estimated that there is about 2500 cases of listeriosis and 500 deaths annually in the United States. During the period of 1998-1999 most of the outbreaks in the US involved consumption of hotdogs with 101 cases of illness and 21 deaths (Donnelly, 2001). Because of serious health consequences, *L. monocytogenes* has been recognized as an important public health problem and has elicited a worldwide response from various sectors including food industries, health agencies, and government bodies with the aim of detecting and controlling infections caused by the presence of *L. monocytogenes* in food (CDC, 2003).

Several outbreaks of listeriosis have been reported in last few years due to consumption of *Listeria*-contaminated foods. Outbreaks of listeriosis have also occurred worldwide. In a study conducted in Northern Ireland, the occurrence of *Listeria* was determined both qualitatively and quantitatively on 513 selected food samples produced over one year at the point of dispatch from various processors. According to this data, *L. innocua*, *L. monocytogenes*, *L. seeligeri* and *L. welshimeri* were the most frequently isolated species (CDC, 2004). Moura

et al. (1993) studied the incidence of *Listeria* spp. in raw and pasteurized milk from a Brazilian dairy plant and identified milk samples positive for *Listeria* spp. Although the largest outbreaks of human listeriosis have initially involved dairy products, numerous recent cases of listeriosis have been linked to meat products (Farber and Peterkin, 1991).

Six major outbreaks occurred in North America between 1979 -1999. The first outbreak was observed in 1979, at least 23 were hospitalized because of consumption of *L. monocytogenes* contaminated lettuce, carrots, and radishes in Boston, MA. The second outbreak was in 1981 in the Maritime Provinces of Canada. This outbreak involved 41 cases and 17 deaths due to the consumption of contaminated coleslaw. And the third outbreak was in Massachusetts in 1983 with a case-fatality rate of 29% associated with contaminated whole and 2% milk. The next outbreak occurred in 1985 in Southern California and Mexican style cheese was implicated as the vehicle of infection (Donnelly, 2001). In one outbreak twenty-five environmental samples from a poultry processing plant in Pennsylvania yielded *L. monocytogenes* resulting in a voluntarily recall of 27.4 million lbs of fresh and frozen ready-to-eat turkey and chicken products on October 12, 2002 (CDC, 2002).

Several major foodborne outbreaks have been documented in North America and Europe. The incidence of *L. monocytogenes* in RTE foods can range from 1-10% (Farber et al., 1996). Another possible high-risk food was vacuum-packed gravad and cold smoked rainbow trout/salmon, which are excellent mediums for the growth of *L. monocytogenes*. Reports have shown that

the occurrence of *L. monocytogenes* in gravid rainbow trout/salmon was 21-27% and in cold-smoked products were 11-14% (Tham et al., 2000). By combining the data from all of the outbreaks, 92.4% of the cases were related to the consumption of contaminated meat and dairy products. Meat ranked first followed by dairy products, produce and seafood (Table 3). Outbreaks of listeriosis in the United States between 1970 and 2002 were listed in the table 2.

The reason for the high prevalence of *L. monocytogenes* in different kinds of foods is partly attributed to its extended distribution in the environment and specific growth conditions, changing human demographics and behavior, and the breakdown of public health measures (Altekruse et al., 1997). Long shelf-life products such as RTE (ready-to-eat) products may also allow growth of *L. monocytogenes* during storage (Vitas et al., 2004).

Year	Food Vehicle	State	Cases	Perinatal Cases (% of Total)	Deaths (% of Total)	Serotype	Reference
1979	Raw Vegetables or Cheese	MA	20	0(0)	3(15.0)	4b	Ho, 1986
1983	Pasteurized Milk	MA	32	7(21.9)	14(43.8)	4b	Fleming, 1985
1985	Mexican-Style Cheese(raw milk)	CA	142	93(65.5)	48(33.8)	4b	Linnan, 1988
1986-1987	Ice Cream, Salami, Brie Cheese	PA	36	4(11.1)	16(44.4)	4b, 1/2b, 1/2a	Schwartz, 1989
1986-1987	Raw Eggs	CA	2	Unknown	Unknown	4b	Schwartz, 1988
1987	Butter	CA	11	Unknown	Unknown	Unknown	Ryser, 1999a
Not Specified	Frozen Vegetables	TX	7	3(42.9)	Unknown	4b	Simpson, 1996
1998-1999	Hotdogs, Deli Meats	22 States	101	Unknown	21(20.8)	4b	Mead, 1999
1999	Pate	CT,MD,NY	11	2(18.2)	Unknown	1/2a	Carter, 2000
2000	Deli Turkey Meat	10 States	29	8(27.6)	7(24.1)	Unknown	CDC, 2000b
2000-2001	Homemade Mexican Style Cheese (raw milk)	NC	12	10(83.3)	5(41.7)	Unknown	CDC, 2001
2002	Deli Turkey Meat, Sliceable	8 North Eastern States	63	3(4.8)	7(11.1)	Unknown	CDC, 2002b
Total			466				

Table 3. A comparative ranking of types of food group vehicles by number of outbreaks and number of cases in United States (CFSAN, 2001).

Type of Food Vehicle	Ranking Order by the Number of Outbreaks or Cases	
	Outbreaks	Cases
Dairy	1	2
Meat	2	1
Seafood	4	4
Produce	3	3

## 1. 2. *Listeria* significance in RTE Foods

### 1. 2.1. Properties of *L. monocytogenes* in relation to food and food processing

The definition of RTE foods was given by FSIS (2003) as “RTE meat and poultry products are products that are in a form that is edible without additional preparation to achieve food safety and may receive additional preparation for palatability or aesthetic, gastronomic, or culinary purposes”. In order to protect human health, emphasis should be given on prevention and elimination of this pathogen from RTE meat and meat products because there is no cooking necessary before consumption. It is very difficult to completely eliminate *L. monocytogenes* from food production facilities as it has the ability to grow in many different processing environments. *L. monocytogenes* can grow in foods



even after packaging because it is a facultative anaerobe (Buchanan et al., 1989). Outbreaks and sporadic cases are predominantly associated with RTE foods. It has the ability to grow at refrigeration temperatures as low as 0.5°C and can even survive freezing (Seeliger and Jones, 1986; Junntila et al., 1988). The growth temperature for *Listeria* is between 1-45°C but optimal growth occurs between 30 and 37°C (Ryser and Marth, 1991). Temperatures above 45°C limit the growth of *L. monocytogenes* and it is destroyed by pasteurization at 71.6 °C in 15 mins (Petran and Zottala, 1989; Lou and Yousef, 1999). However, the organism can multiply due to re-contamination of food after heat treatment followed by temperature abuse (Buncic et al., 1990; Zaika et al., 1989). It has been shown that cold-smoking can eliminate *L. monocytogenes* between 17.1 to 21.1°C, whereas the survival of this bacterium has been observed at temperatures from 22.2 °C to 30 °C (Liv, 2000). The incidence of *L. monocytogenes* increases after chilling and cutting of meat products. Food products that are highly processed, having extended shelf-life at refrigeration temperatures are capable of supporting the growth of *L. monocytogenes* and foods that were consumed without further cooking are most associated with the transmission of this organism. *L. monocytogenes* has the ability to grow in a wide variety of processing environments, this property and tolerance to common preservation agents makes *L. monocytogenes* a particular concern as a contaminant during processing (Mclauchlin, 1996). Contamination can occur even during the very last step of processing, such as packaging (Tham et al., 2000).

A 15.5% incidence level of *L. monocytogenes* was found in RTE chicken and turkey products as reported by Rijpens et al. (1996). In 1993, Wang et al reported that the incidence of this pathogen in one brand of deli turkey meat products was as high as 71%. Deli turkey meat from one large processor caused twenty nine illnesses, including four deaths and three miscarriages/stillbirths due to contamination with *L. monocytogenes* (CDC, 2000).

Based on the strategies given by the National Advisory Committee on Microbiological Criteria for foods (NACMCF, 1991), the most effective method for controlling *L. monocytogenes* is implementation of the Hazard Analysis Critical Control Point system (HACCP) and process control strategies

### **1. 2. 2. Prevalence of *L. monocytogenes* in raw materials**

In terms of food safety, the detection of *L. monocytogenes* in meat is very important. *L. monocytogenes* can grow on both raw and cooked meat and even at refrigeration temperatures. On the other hand, during processing of raw meat into meat products *L. monocytogenes* can be introduced into the environment where RTE product is produced. The extent of cross-contamination largely depends on the training of personnel and on general hygienic measures as much as on the food processing parameters (Yucel et al., 2005). There is a chance of finding multiple clonal types of *L. monocytogenes* on contaminated products from a single processing plant (Tham et al., 2000).

The prevalence of *L. monocytogenes* in 13 dried sausage processing plants and their products was studied by Thevenot et al. (2005) and reported that

71.6% of collected samples were shown positive for the presence of *L. monocytogenes*. In that, 15% were positive before the beginning of the working day and 47.3% during working day. However, a decrease in the contamination rate was observed during the drying stage. Even if the contamination rate is usually low, manufactures have to follow the regulations requiring a zero-tolerance for *L. monocytogenes* on RTE meats.

Although dairy products have been a major source of contamination, raw or re-contaminated products of animal or vegetable origin may serve as the vehicle of transmission for this pathogen (Yucel et al., 2005). Live animals are known to harbor *L. monocytogenes* in feces and are considered as a source of contamination during slaughter (Buncic, 1991). The main place where *L. monocytogenes* can originate might be a primary production step, that is, farm where domestic animals were raised. Many investigations were undertaken to examine the prevalence of *L. monocytogenes* in fecal samples from cattle. In a study done by Skovgaard and Morgen (1988) they isolated *L. monocytogenes* from silage and alkalized straw in 62% and 67% of investigated samples and the frequency of *L. monocytogenes* in cattle feces was 52%. And they also collected 67 samples of minced beef from retail stores and reported that the 28% of the samples tested showed the presence of *L. monocytogenes*.

### **1. 3. Factors affecting growth and survival of *L. monocytogenes*.**

#### **1. 3. 1. Temperature**

High mortality rates associated with *L. monocytogenes* are mainly observed with young, old, pregnant women and immuno-compromised patients (Dykes, 2003). Several listeriosis cases were reported from consumption of raw, inadequately heated, or re-contaminated cooked foods. In order to avoid listeriosis and to ensure food safety in the RTE meat and poultry products it is very important for the processed meat industry to evaluate their processes to meet lethality performance standards (Murphy et al., 2004).

*L. monocytogenes* can grow at wide range of temperature (3 °C to 45°C) and the optimum growth temperature is between 30°C and 37°C under laboratory conditions (Petran and Zottola, 1989). Many factors influence the heat resistance of *L. monocytogenes* including time and temperature of incubation or the formation of a thermostable membrane resulting in increased thermotolerance and expression of heat shock proteins. Heat shock proteins are set of proteins synthesized by microorganisms when they are exposed to sublethal temperatures. These heat shock proteins protect organisms from the toxic effects of heat and other stresses. Heat shock increases the heat resistance capacity of microorganisms (Knabel et al., 1990). A study done by Farber and Brown (1990), demonstrated that the optimum temperature for inducing heat tolerance of *L. monocytogenes* was between 44°C and 48°C. Obtaining thermotolerance depends on the temperature that the heat shock is done, the physiological state

of the cells and the incubation temperature of the cells (Lindquist, 1986). An increased thermotolerance in *L. monocytogenes* was observed in cells which had been heat shocked in broth at 43°C for 5, 30, or 60 mins (Knabel et al., 1990).

The possible explanation for the incidence of *L. monocytogenes* in the RTE products is the survival of *L. monocytogenes* to the initial cooking process, post-process contamination and recuperation and growth of injured *L. monocytogenes* during storage (Bersot et al., 2001). Cooking the product to an internal temperature of 70°C for 2 minutes is adequate to destroy *L. monocytogenes* (Mackey and Bratchell, 1989). Bersot et al. (2001) also showed that cooking the product to an internal temperature of 74°C and a careful handling and storage eliminates microorganisms including the *L. monocytogenes*.

In solidly frozen foodstuff microbial growth not is possible, although some organisms can grow at refrigeration temperatures. In general *L. monocytogenes* is a psychrophile thought to have enzymes which are active at low temperature and which have high concentration of unsaturated fatty acids in the cell membrane which protects the cell from freezing. The psychrotrophic properties of *L. monocytogenes* are dependent on the structural integrity of the cell and metabolism at low temperature is stimulated by a cold-resistant sugar transport system. Low temperature has been described to increase the production or inactivation of an extracellular hemolysin and also the virulence of *L. monocytogenes* (Junttila et al., 1988).

### 1. 3. 2. pH

*L. monocytogenes* grows best in the pH range 4.1 to 9.6 with optimal growth occurring at neutral to slightly alkaline pH values (Seeliger et al., 1987). The exact minimum pH that allows the growth of *L. monocytogenes* is not clear. But the factors such as temperature of incubation, nutrient composition of growth substrate, water activity and presence and amount of NaCl and other salts or inhibitors play a role in deciding minimum growth pH of a bacterium. At pH 4.5 growth of four strains of *L. monocytogenes* was observed when incubated at 30°C for 30 days, but no growth was observed at pH 4.0 or lower (James, 2000). Konstantinos et al. (2004) demonstrated that the minimum pH that permits the growth of *L. monocytogenes* in tryptic soy broth or on tryptic soy agar was 4.45 and 5.10 respectively. Even restriction of oxygen can enhance the growth of this organism at pH 4.5. The minimum growth of *L. monocytogenes* was observed at pH 4.66 in 60 days at 30°C, pH 4.83 at 10°C, but no growth at 5°C at pH 5.13. The pH is also affected by various acids. At the same pH, the antimicrobial activity of different acids are as follows, acetic acid > lactic acid > citric acid > malic acid > HCl (James, 2000). The ability of *L. monocytogenes* strains to grow on bologna and summer sausages under the same water activity with different pH (higher in bologna than summer sausage) was studied by Dykes (2003) and demonstrated that the ability of *L. monocytogenes* on bologna and inability to grow on summer sausage under similar storage conditions. This parameter (pH) was the major intrinsic factor preventing the growth of *L. monocytogenes* on summer sausage or comparable products.

### 1. 3. 3. Water activity ( $a_w$ )

Another important mechanism that is responsible for slowing the growth rate of microorganisms is related to the microenvironment surrounding growing colonies. Apart from temperature, pH, and other factors including food structure, microbial interaction, physiological state of the cell, and inoculum size, the growth of *L. monocytogenes* is also affected by water activity ( $a_w$ ). Unlike many other pathogens it has the ability to grow at low water activity (Konstantinos et al., 2004). The minimum water activity that permits the growth of *L. monocytogenes* was determined by using three humectants (glycerol, sucrose, and NaCl) and brain heart infusion (BHI) at 30°C of incubation, and found that the serotypes 1, 3a, and 4b of *L. monocytogenes* grow at  $A_w$  of 0.90 with glycerol, 0.93 with sucrose, and 0.92 with NaCl. In tryptic soy broth with a pH 6.8 at 30°C incubation the minimum  $A_w$  that permits the growth of *L. monocytogenes* was 0.92 (James, 2000). Survival of *L. monocytogenes* was observed but it did not grow at 4°C with a water activity of 0.79-0.86 (Johnson et al., 1988). The exceptional characteristics of *L. monocytogenes* to grow at low water activity, provides it with a perfect environment to survive and multiply in ready-to-eat meat products.

### 1. 3. 4. Atmosphere

*L. monocytogenes* can grow irrespective of the presence or absence of oxygen. It is a facultative anaerobe. However, it has been observed that this organism's growth is enhanced in an atmosphere containing 5-10% CO<sub>2</sub>. One of

the major concerns in the food industry is that *L. monocytogenes* has the ability to grow in vacuum-packaged temperature-abused atmospheres. Modified atmosphere packaging (MAP) methods have been examined as a possible control for growth and survival of this pathogen. The gasses used in MAP are most often combination of oxygen (O<sub>2</sub>), nitrogen (N<sub>2</sub>), and carbon dioxide (CO<sub>2</sub>). A modified high-CO<sub>2</sub> packaging atmosphere has recently depicted a reduced growth trend of this pathogen in food products. The bacteriostatic effect is obtained by the combination of decreased O<sub>2</sub> and increased CO<sub>2</sub> dissolved in the water phase of a food matrix (Jydegaard-Axelsen et al., 2004). Inhibition of *L. monocytogenes* was observed in turkey roll slices that were packed in an atmosphere containing 70% CO<sub>2</sub> and 30% N<sub>2</sub> (Farber and Daley, 1994).

#### **1. 4. Government regulations**

Approximately 6.5-8.1 million cases of diarrheal diseases and 9000 deaths occur annually in USA due to the foodborne pathogens such as *Salmonella*, *Campylobacter Jejuni*, *E. Coli* O157:H7, *Y. Enterocolitica* and *L. monocytogenes* including certain parasites. These pathogens are not only causing diseases, infections, and much human suffering but also affecting the economy by lowering productivity, increasing medical costs, litigation and loss of business for the food industry (FSIS, 2004).

During 1980's *L. monocytogenes* became as a foodborne pathogen of public concern. In the 1990's, state health departments and the CDC investigated an outbreak of foodborne illness in which hotdogs, and possibly deli (luncheon)



meats, were implicated and they found the same strain of *L. monocytogenes* from both an opened and previously unopened package of hotdogs manufactured by a single plant. There were 101 illnesses, 15 adult deaths, and 6 stillbirths or miscarriages were associated with this outbreak.

Based on the information from different outbreaks, combined with other food safety investigations, it is understood that lack of control measures such as an appropriate HACCP (Hazard Analysis Critical Control Point) plan and Sanitation Standard Operating Procedures (Sanitation SOP) in food processing industries (FSIS, 2004) are the major responsible factors for outbreaks of listeriosis. The fatalities associated with this organism prompted FDA and FSIS (Food Safety and Inspection Service) of the U.S. Department of Agriculture to establish zero tolerance for this pathogen in RTE foods, because the infective dose is still unknown. In May 1999, FSIS established strategies and directives for controlling *L. monocytogenes* in RTE meat products (FSIS, 1999).

According to the FSIS Directive: 10,240.3 (Anonymous, 2002), instructions are given to specially trained people on when to sample ready-to-eat meat and poultry products produced in processing establishments. The products are divided into three categories such as high, medium, and low risk RTE meat products. High and medium risk products includes deli-type (which are not sliced in the establishment or will be sliced at retail), hot-dog type products, and deli- and hotdog-type products have not been formulated or not produced and distributed under conditions validated to prevent the growth of *L. monocytogenes*. Low risk products include deli-type products (which are sliced in

the establishment or will be sliced at retail), and hotdog type products that have been formulated or are produced and distributed under conditions that validate to prevent the growth of *L. monocytogenes*. The main purpose of this directive is to produce safe, wholesome food and to control *L. monocytogenes*.

To make it a most effective strategy to control *L. monocytogenes*, FSIS also established three alternatives. The alternative one includes, employing both post-lethality treatments to control *L. monocytogenes* and growth inhibitor which suppresses the growth of microorganism on RTE meat and poultry products. The main reason in using antimicrobials is that if *L. monocytogenes* survives the post-lethality treatment, then the antimicrobial can prevent its further growth on the finished product. Alternative two includes employing either a post-lethality treatment or a growth inhibitor for *L. monocytogenes* on RTE meat and poultry products. Finally the third alternative includes employing only sanitation measures to control *L. monocytogenes*. The incentives for processors to accommodate the additional efforts involved with alternative 2 or 1, is less product sample testing (Anonymous, 2003; FSIS, 2004). Present research along with risk assessment programs will help in preventing *L. monocytogenes* in RTE products and reduce the number of illness caused by this organism. USDA recall classifications are shown in the table 4.

Table 4. USDA Recall Classification. (FSIS, 2002)

Class I	This is a health hazard situation where there is a reasonable probability that the use of the product will cause serious, adverse health consequences or death.
Class II	This is a health hazard situation where there is a remote probability of adverse health consequences from the use of the product.
Class III	This is a situation where the use of the product will not cause adverse health consequences

## **1. 5. Effectiveness of inhibitory compounds in inhibiting the growth of *L. monocytogenes***

### **1. 5. 1. Chemical preservatives**

The important function of preservatives is to kill, delay, or prevent the growth of microorganisms (Gould, 1996). Reduction of the losses attributed by spoilage organisms such as *L. monocytogenes* increase the productivity by improving the shelf life of the product. Several outbreaks on listeriosis increased the need to know more information about intrinsic factors (atmosphere,  $A_w$ , pH) of the food and environmental parameters that influences the growth of *L. monocytogenes* in contaminated foods (Razavilar and Genigeorgis, 1998).

In recent years additives have been used not only as preservatives but also as color and water retention enhancers (Lemay et al., 2002). Preservatives are defined by Adams and Moss (2000) as “substances capable of inhibiting,

retarding or arresting the growth of microorganisms or of any deterioration resulting from their presence or of making the evidence of any such deterioration". Preservatives can kill the target organism (bactericidal) or can simply prevent their growth which means bacteriostatic. Adams and Moss (2000) said that adding lower concentrations of preservatives tend to act as bacteriostatic and use of higher concentrations of preservatives is now more restricted and controlled. For this reason, chemical preservatives are useful to control low levels of contamination.

Even though sulfurdioxide ( $\text{SO}_2$ ) has disinfecting properties, it has the ability to destroy the vitamin thiamine in foods. Therefore the use of sulfurdioxide as a preservative in meat and meat products has now been prohibited (Adam and Moss, 2000). The use of organic acids combined with bacteriocins or other natural antimicrobials inhibits the bacterial growth even when used at low concentrations (Samelis et al., 2005). The use of acetic, citric, and lactic acids to inhibit the growth of *L. monocytogenes* is well known (Ahamed and Marth, 1989).

The active agent in sodium nitrite and its antimicrobial activity increases with decreasing pH (Adam and Moss, 2000). But many studies have shown that nitrites can have carcinogenic effect, so its use is limited. Sodium nitrate ( $\text{NaNO}_2$ ) and sodium chloride ( $\text{NaCl}$ ) are two most frequently used chemicals in the meat industry and have exhibited antilisterial attributes. Buchanan et al. (1988) said that Sodium nitrite ( $\text{NaNO}_2$ ) has the bacteriostatic effect at pH of 6.0 and at temperature  $5^\circ\text{C}$ . At a temperature of  $20^\circ\text{C}$  and pH 5.3,  $50\mu\text{g/ml}$  of  $\text{NaNO}_2$  would prevent the growth of *L. monocytogenes* for up to 21days (McClure et al., 1991).

The growth can be completely inhibited with NaCl when combined with other chemicals such as sodium lactate (Chen and Shelef, 1991). The combinations of NaNO<sub>2</sub> and NaCl (120-150 ppm NaNO<sub>2</sub> and 3% NaCl) prevent the growth of *L. monocytogenes* in fermented sausages (Johnson et al., 1988; Junttila et al., 1989).

Effect of 0.3% sodium propionate (NaP), Potassium sorbate (KS), and sodium benzoate (NaB) on the growth of *L. monocytogenes* was studied by El-Shenawy and Mart (1988, 1989) and found that the effectiveness of KS, NaP, and NaB were increasing with decreasing pH and temperature. And they also observed that the growth of *L. monocytogenes* in a pH range from 5.85 to 6.10 with 12% salt at the temperature 8 to 30°C. 6-9 days of shelf life extension was observed in cottage cheese made with Microgard which is a fermented and pasteurized skim milk-based product containing various anti microbial additives such as acetic acid, propionic acid, and proteinaceous inhibitor (Salih et al., 1990).

Weak organic acids such as sorbic and benzoic acids are widely used in food industries. Incorporation of organic acids into the formulation of RTE processed meats may reduce the growth of *L. monocytogenes*. The growth of *L. monocytogenes* was prevented in vacuum-packaged frankfurters treated with 0.5% lactic and acetic acid kept at 5°C for 90 days storage (Palumbo and Williams, 1993).

*L. monocytogenes* has the ability to grow in high salt concentrations (Razavilar and Genigeorgis, 1998). The survival of *L. monocytogenes* was

observed in raw ground beef at pH 5.5 when stored aerobically for 15 days even in the presence of additives such as sodium lactate, kappa-carrageenan, sodium erythorbate, and combination of sodium alginate/lactic acid/calcium carbonate (Harmayani et al., 1993).

### **1. 5. 2. Sodium lactate**

Consumers prefer safe and nutritionally healthy foods which do not cause allergies, infections, diseases and any other health complications. Their demand has currently been towards foods which are “natural”, higher in quality, free of additives, safe and convenient to use (Rhodehamel, 1992). Hence, it is very important to develop natural ingredients having antioxidative and antimicrobial properties.

Sodium lactate is a food additive, and a natural component of muscle tissue, and therefore is naturally present in meat. It is permitted by the USDA-Food Safety and Inspection Service (USDA-FSIS) as a natural preservative and the permitted level is 3g/100g of meat (Samelis et al., 2001). Up to 4.8% by weight of the total formulation is approved by the FDA in fully cooked meat and poultry as a flavoring agent and antimicrobial (FDA, 2000). This is produced naturally by microbial fermentation and it is a sodium salt of lactic acid. The main advantage of sodium lactate is that it can be used to control the growth of certain microorganisms such as *Clostridium botulinum* and *L. monocytogenes* during storage. It also improves the flavor and processing parameters and extends the shelflife of meat products (Choi and Chin, 2003; Bloukas et al., 1997). The

addition of sodium lactate delayed the production of toxin by *Clostridium botulinum* in turkey meats (Mass et al., 1989). Lamkey et al. (1991) reported that addition of sodium lactate not only inhibited the growth of *L. monocytogenes* but also extended the shelf-life by more than two weeks. Antimicrobial activity of sodium lactate is similar to potassium sorbate and sodium benzoate. Sodium lactate delayed the lag phase for the growth of *L. monocytogenes* without affecting the quality of sausages (Choi and Chin, 2003). The addition of sodium lactate may also improve various quality attributes of meat. It improves color, flavor, tenderness, juiciness and cooking yield of beef and other meat and also has an inhibitory effect on lipid peroxidation (Sallam and Samejima, 2004). Sodium lactate is currently accepted as Generally Recognized as Safe (GRAS) for use as an emulsifier, flavor enhancer, flavoring agent, humectant and pH control agent at levels not exceeding current good manufacturing practices (Brewer et al., 1991). Increased cooking yields were observed with increasing sodium lactate level in cooked, vacuum-packaged beef top rounds due to a combination of the humectant property of sodium lactate and increased level of sodium ions (Papadopoulos et al., 1991). It has no effect on pH, textural properties and cooking loss of low-fat frankfurters which are produced with 3% sodium lactate (Bloukas et al., 1997). The possible mechanism of sodium lactate not only includes lowering the water activity but also other properties such as cytoplasmic acidification, specific anionic effect, and chelating action. Sub-optimum temperatures increase the antimicrobial effects of sodium lactate which is useful in controlling the growth of *L. monocytogenes* at refrigerated

temperatures (De Wit and Omboust, 1989). An increase in fat content, decrease in moisture content, and/or a decrease in temperature increases the effectiveness of sodium lactate against *L. monocytogenes*. In preventing the growth of *L. monocytogenes*, this property could be very useful in RTE food products which are held at refrigeration temperature. In contrast, no significant difference was found in low-fat frankfurters treated with and without sodium lactate (Hu and Shelef, 1996; Chen and Shelef, 1991).

### **1. 5. 3. Sodium diacetate**

The concern over negative health effects of food preservatives increases pressure on food industry to replace chemically synthesized additives with natural ones for assurance of food safety and extension of shelflife. Diacetyl is produced by yeasts and lactic acid bacteria and approved as GRAS (Generally Recognized as Safe) (Newberne et al., 2000). Among the substances that come from microbial origin, diacetyl seems to be harmless because of absence of toxicity against humans and animals (Lanciotti et al., 2003). Up to 0.25% by weight of the total formulation is approved by the FDA (FDA, 2000).

Even though the use of 0.2% or higher concentration of sodium diacetate suppresses the growth of *L. monocytogenes*, higher levels of its concentration results in unacceptable off-flavors in finished meat and poultry products (Shelef and Addala, 1994). However, the use of sodium diacetate in combination with lactates increases the antimicrobial activity by reducing the water activity of the products. The main antimicrobial ingredients present in this combination are



lactic acid and acetic acid (Stekelenburg, 2003). Gram-positive bacteria were more resistant whereas Gram-negative and yeasts were sensitive to diacetyl (Lanciotti et al., 2003). The effect of diacetyl with nitrite, lactate, or pediocin on the viability of *L. monocytogenes* in turkey slurries was studied by Schlyter et al. (1993). According to their study, diacetate at levels of 0.5% at 25°C or 0.3% at 4°C were effective against *L. monocytogenes*. The different concentrations of nitrite, lactate and pediocin were ineffective at 25°C in controlling the growth of *L. monocytogenes*. But pediocin in combination with 0.5% diacetate, lactate (0.5%), diacetate and nitrite with 0.5% diacetate exhibited greatest listericidal activity at 25°C. The addition of 2–3% of a solution containing mixtures of 56% potassium lactate and 4% of sodium diacetate showed inhibitory action against *L. monocytogenes* and *L. sake* in the frankfurter-type sausages during storage at 4°C. The synergistic effect of potassium lactate and sodium diacetate inhibited the development of *L. monocytogenes* and resulted in an increase in shelflife of 75-125% in frankfurter type sausages (Stekelenburg, 2003).

#### **1. 5. 4. Sodium acetate**

Sodium acetate has also exhibited an antimicrobial effect against *L. monocytogenes* in a variety of meat and poultry products. Up to 0.25% by weight of total formulation is also approved by the FDA (FDA, 2000). An uncured turkey product when surface treated with 6% sodium acetate solution and stored for 18 days at 28°C showed the inhibition of *Clostridium botulinum* neurotoxin production (Miller et al., 1992).

### 1. 5. 5. Plant essential oils

Food safety is an increasingly important public health issue. Approximately two million people died annually from diarrhoeal disease worldwide (WHO, 2002). The meat industry must now use chemicals as preservatives and as a means of preventing microorganisms in the food products. But consumer demand over food that is free from chemicals and microorganisms has prompted an increased interest in more natural preservatives. It is very important to find safe effective replacements for chemical preservatives. Essential oils are obtained from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots. These essential oils are aromatic oily liquids. The methods in which we can obtain essential oils are expression, fermentation, effleurage, or extraction. But the most commonly used method to extract essential oils commercially is steam distillation (Burt, 2004). Essential oils have been shown to exhibit antimicrobial, antiviral, antimycotic, antitoxigenic, antiparasitic and insecticidal properties (Burt, 2004; Shelef, 1983; Deans and Ritchie, 1987; Aureli et al., 1992; Lis-Balchin, 1987; Smith-Palmer, 2001). The decrease in the pH of the product increases the effectiveness of essential oils (Burt, 2004). The use of sodium lactate together with the essential oil of mustard were a most effective combination of preservatives and its effectiveness can be further increased by combining with other antimicrobials due to a synergistic effect (Lemay et al., 2002). The efficacy of essential plant oils (thyme, clove, pimento, rosemary, and sage) as antimicrobial agents against *L. monocytogenes*

was studied in hotdogs by Singh et al. (2003). Their results revealed that no particular strains were most susceptible or most resistant to the antimicrobial action of the various essential oils. Of the essential oils tested, thyme oil was the most inhibitory against all strains of *L. monocytogenes*. The effectiveness of thyme essential oil depends on the amount of the fat present in the product. It exhibited antimicrobial activity even at 1ml/l concentration in nonfat hotdogs. The increase in fat content has been shown to appreciably decrease the antimicrobial activity of thyme essential oil against *L. monocytogenes* (Smith-Palmer, 2001). There is a significant reduction in the population of *L. monocytogenes* from 0.88 to 0.99 log<sub>10</sub> CFU/g in hotdogs treated with clove essential oil with 1ml/l concentration (Singh et al., 2003). The possible mechanism of antimicrobial activity of essential oils against microorganisms could be a change in the cell membrane structure of microorganisms and causing leakage of nutrients and enzymes (Cox et al., 2000). As the concentration of clove essential oil increases, there is no significant further reduction in the population of *L. monocytogenes*. The main reason for this behavior could be interference of various food components with essential oils and protective effect of fat and protein, reduction of water availability. The longer the contact time between the microorganisms and the essential oils the more it helps to overcome the protection offered to the cell wall of microorganism by excess fat present in the hotdogs (Singh et al., 2003). At the levels of 5 – 20 µl g<sup>-1</sup> of eugenol and coriander, clove, oregano and thyme oils were found to be effective against *L. monocytogenes*, but mint and

cilantro were not effective in products with high levels of fat (Burt, 2004; Tassou et al., 1995; Gill et al., 2002).

Since the sensory quality of the product is very important, the use of essential oils as a preservative and antimicrobial agent is limited. Eventhough, these essential oils have more advantage and are recognized as GRAS, some essential oils such as eugenol, menthol and thymol are known to cause irritation of mouth tissue and some times cause allergies to the people who use them more frequently (Burt, 2004). Further food safety studies may be required to find out the effective ways to use these essential oils.

## **1. 6. Smoke flavorings/extracts**

### **1. 6. 1. Traditional smoking**

The Traditional method of smoking which involves several steps, inhibits the spore formation, growth of many bacteria, and fungi as well as viral activity. This method involves salting and smoking which results no chance of survival of *Listeria* (Daun, 1979). Cold smoking and hot smoking are two traditional smoking methods used mainly in processing of RTE meat products.

When cod fillets subjected to cold smoking and were inoculated with three different levels of *L. monocytogenes*, there was a very little growth of *Listeria* in fillets inoculated with a low level inoculum (Dillon and Pate, 1993). Usually cold smoking is conducted at  $\leq 28^{\circ}\text{C}$  of temperature which provides a favorable environment for microbial growth (Dillon and Patel, 1993; Guyer and Jemmi,

1991). Where as hot smoking is conducted at above 60° C. Many studies have indicated that cold smoked or hot smoked products present a risk for human listeriosis (Rorvik, 2000). Some smoking techniques include flue gas smoking in which they use traditional kilns. The disadvantage of this flue gas smoking is that it generates polyaromatic hydrocarbons (PAH) emissions to air and to water in the form of waste water. After processing with this method, the products end up with these polyaromatic hydrocarbons, which are carcinogenic compounds. Hence, it is not favorable from an environmental point of view (Hattula et al., 2001). Usually seafoods are traditionally smoked for preservation purposes. Smoking of seafoods may be done either by cold smoking or by hot smoking. Even though hot smoking should have been a bactericidal process, most of the *L. monocytogenes* contamination was from hot smoked products. It has been suggested that post-process contamination could be contributing factor.

In traditional smoking technique, smoke is generated from the incomplete burning of wood which leads to the production of polyaromatic hydrocarbon compounds which infiltrates the surface of food products incubated during the process (Guillen et al., 2000). Foods produced by hot and cold smoking methods do not have bactericidal activity against *L. monocytogenes*, and therefore such products may pose a public health hazard in terms of listeriosis (Dillon and Patel, 1994).

## **1. 6. 2. Commercial smoking**

### **1. 6. 2. 1. Liquid smoke / purpose of smoking**

The main purpose of smoking meat products with liquid smoke is that it not only acts as a coloring and flavoring agent but also it has antibacterial and antioxidative properties (Coronado et al., 2002). The various phenolic compounds present in liquid smoke lowers the pH and destroys the walls of bacterial cells (Pszdzola, 1995). The use of liquid smoke in the production of meat products was increased 30% from 1973 to 1976 in the U.S. (Sink and Hsu, 1978). There are several methods for applying liquid smoke. Liquid smoke can be directly added to the meat batter, or dipping the product in to the liquid smoke solution, or by spraying the smoke solution over the product.

Hattula et al. (2001) studied whether a liquid smoke process could replace the traditional cold smoking process of rainbow trout fillets or not. They showed that liquid smoked fillets were as good as those smoked traditionally and the emission of polyaromatic compounds to the environment decreased compared to the traditional smoking process. Lipid oxidation of lipids either from meat muscles or from animal fat is a large problem causing deterioration of the meat products (Allen and Allen, 1981). Lipid oxidation leads to loss of essential fatty acids, fat-soluble vitamins, and also causes undesirable changes in the flavor, color, texture, and functional properties of products (Shahidi, 1994; Addis, 1986; Pearson et al., 1983), which finally affects the quality of the product and its shelflife (Gray and Pearson, 1994). Many studies have revealed that smoke

flavorings also have antioxidant properties. Guillen and Cabo (2004) demonstrated that liquid smokes have shown antioxidant activity on the lipids of pork adipose tissue and increased the oxidative stability of the samples. However, Sink and Hsu (1978), suggested that franks processed with an external liquid smoke treatment were less tender, whereas stable color was attributed to franks in which liquid smoke was mixed in to the emulsion

#### **1. 6. 2. 2. Composition of smoke**

Liquid smoke is rich in phenolic compounds but poor in carbonyl derivatives, causing changes in cohesiveness, springiness and gumminess of the products (Martinez et al., 2004). All palatability properties were significantly correlated with phenol content of the smoked product (Sink and Hsu, 1978). The possible mechanism could be attributed to the phenolic compounds forming hydrogen bonds with water in meat products and influence the water holding capacity and springiness. The increase in the water content decreases the cohesiveness and hardness of the meat products (Rongrong et al., 1998). Another mechanism is the chemical reaction, or interaction between smoke constituents such as carbonyl compounds and proteins resulting in a greater firmness to meat products (Guillen and Ibargoitia, 1998). Carbonyl compounds may have less capacity in establishing hydrogen bonds with water compared to phenolic compounds (Martinez et al., 2004).

Characterization of the components of salty smoke flavor preparation was done by Guillen and Ibargoitia (1996) and concluded that phenol derivatives

including syringol and syringol derivatives, guaiacol and guaiacol derivatives were the main components responsible for smoke flavor. Smoking of food is one of the oldest methods used from 10,000 years. Sensory active components such as phenol derivatives, carbonyls, organic acids and their esters, lactones, pyrazines, pyrroles and furan derivatives are responsible for many of the aromatic properties of meat products (Simko, 2002).

Phenols present in liquid smoke not only contribute to the color and flavor but also have antioxidant and antimicrobial properties (Coronado et al., 2002). Alcohols serve as volatile compounds and organic acids have minor preservative effect but play an important role in skin formation and protein coagulation on the surface of sausages. Carbonyls are largely responsible for the aroma and flavor of smoked meats but hydrocarbons have been shown to be carcinogenic (Radecki et al., 1978).

### **1. 6. 2. 3. Production of smoke**

The yield of smoke, its compositions, and its organoleptic properties are influenced by several parameters such as the nature of the wood, the temperature of the process, the amount of oxygen present during the smoke generation, the moisture content of the wood and the wood particle size (Guillen and Ibargoitia, 1996).

Liquid smoke can be prepared by thermal pyrolysis of hardwood sawdust when there is limited access of oxygen (Simko, 2002). Smoke is captured in distilled water in an absorption tower and filtered until the proper concentration is



reached. The final filtration will be done to remove hydrocarbons (Guillen and Ibargoitia, 1996; Guillen and Manzanos, 2002). The obtained aqueous smoke flavoring has a gold color and distinctive flavor. Smoke is given off when the internal moisture content approaches 0 and temperature rises rapidly to 570 - 750°F (Simko, 2002). Aqueous smoke flavoring can be prepared by using *Vitis vinifera L shoots* and *Fagus sylvatica L* wood. *Fagus sylvatica L* is rich in compounds that give smokey flavors whereas *Vitis vinifera L* shoots are rich in a compound that gives sweet, burnt, and pungent flavor (Guillen and Ibargoitia, 1999).

#### **1. 6. 2. 4. Inhibitory nature of liquid smoke**

To maintain food safety and increase the shelflife of food products, food manufacturers are using different combinations such as salting, smoking, vacuum packaging, and storage at chilled temperatures during and after processing.

Smoke extracts/condensates are very active against Gram-positive and Gram-negative bacteria (Asita and Campbell, 1990) and have shown some antimicrobial effects (Niedziela et al., 1998). The main components in the liquid smoke are acids, which act against microorganisms. Phenolic compounds are also present and have the highest antimicrobial and flavor activities. Next to phenolic acids, the synergistic effect of acetic acid and isoeugenol are believed to have activity against *L. monocytogenes*. Liquid smoke was effective in reduction of 99.9% of *L. monocytogenes* on frankfurters (Messina et al., 1988).

At a phenolic concentration of 20 ppm and at 4°C, no growth of *L. monocytogenes* was observed on smoked fish products using liquid smoke (Thurette et al., 1998). The minimum inhibitory effect of several commercial preparations of smoke wood concentrates against spoilage and pathogenic microorganisms was studied by Susen (1998). Based on their study they reported that phenols are the main components responsible for the antimicrobial activity of smoke and they also reported the predominant components in liquid smoke and the type of liquid smoke that play an important role in reducing the growth of microorganisms. In many studies it has been shown that liquid smoke can also inhibit the growth of molds. Phenolic components in the liquid smoke have been shown to be responsible for antifungal properties and inhibited aflatoxin formation (Toth and Potthast, 1984; Wendorff et al., 1983).

#### **1. 6. 2. 5. Advantages and disadvantages of liquid smoke**

Smoke flavorings play an important role in the sensory properties of food (Guillen and Ibargoitia, 1996). Texture is the most important determinant of organoleptic quality of meat products and products with poor texture will often be rejected (Schwanke et al., 1996). The interaction between smoke and food components might influence the textural properties of smoked meat products. Martinez et al. (2004) conducted texture profile analysis of meat products treated with commercial liquid smoke flavorings. Of the two commercial liquid smoke flavorings tested, one that was lower in carbonyl compounds modified the cohesiveness, springiness, and gumminess of the loin, and the hardness,

fracturability, and springiness of the bacon. The other liquid smoke flavoring which was rich in carbonyl compounds, caused changes in all texture parameters of bacon, and springiness and gumminess of the loin and. The components in liquid smoke such as formaldehyde, acetic acid, creosote, and high-boiling phenols have exhibited bactericidal properties and meats, which are processed with smoke, can produce more desirable flavor and color (Messina et al., 1988). Since it has several advantages over the traditional smoking methods, the use of liquid smoke flavoring is becoming increasingly common in the meat industry especially in RTE meat products (Pszczola, 1995).

## 1. 7. References

- Adams, R. M., and M. O. Moss. 2000. Food microbiology. University of Surrey, Guildford, UK.
- Addis, P. B. 1986. Occurrence of lipid oxidation products in food. *J. Food Chem & Toxicol.* 24:1021-1030.
- Ahamad, N., and H. E. Marth. 1989. Behavior of *Listeria monocytogenes* at 7, 3, 21 and 35°C in tryptose broth acidified with acetic, citric, or lactic acid. *J. Food Prot.* 52:688-695.
- Allen, C. E., and E. Allen. 1981. Some lipid characteristics and interactions in muscle foods-a review. *J. Food Technol.* 35:253-257.
- Altekruse, F. S., M. L. Cohen, and D. L. Swerdlow. 1997. Emerging foodborne diseases. Center for Disease Control and Prevention, USA.
- Anonymous. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; Final rule. Fed. Reg. 68:34207-34254.
- Anonymous. 2002. Microbial sampling of ready-to-eat (RTE) products for the FSIS verification testing program. FSIS Directive 10,240.3.
- Asita, A. O., and I. A. Campbell, 1990. Antimicrobial activity of smoke from different woods. *Letf. Appl. Microbiol.* 10:93-95.
- Aureli, P., A. Costantini, and S. Zolea. 1992. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. *J. Food Prot.* 55:344-348.
- Bersot, S. L., M. Landgraf, B. D. G. M. Franco, and M. T. Destro. 2001. Production of mortadella: behavior of *Listeria monocytogenes* during processing and storage conditions. *Meat Sci.* 57:13-17.
- Bloukas, G. J., E. D. Paneras, and G. C. Fournitzis. 1997. Sodium lactate and protective culture effects on quality characteristics and shelf-life of low-fat frankfurters produced with olive oil. *Meat Sci.* 45:223-238.

- Brewer, M. S., K. F. Mckeith, E. S. Martin, W. A. Dallmier, and J. Meyer. 1991. Sodium lactate effects on shelf-life, sensory characteristics of fresh pork sausage. *J. Food Sci.* 56:1176-178.
- Buchanan, R. L., G. H. Stahl, and C. R. Whiring. 1989. Effects and interaction of temperature, pH, atmosphere, sodium chloride and sodium nitrite on growth of *Listeria monocytogenes*. *J. Food Prot.* 53:844-851.
- Buchanan, R. L., G. H. Stahl, and C. R. whiting. 1988. Effects of interactions of temperature, pH, atmosphere, sodium chloride, and sodium nitrite on the growth of *Listeria monocytogenes*. *J. Food Prot.* 15:247-264.
- Buchanan, E. R., N. E. Gibbons, S. T. Cowan, J. G. Holt, J. Liston, R. G. E. Murray, C. F. Niven, A. W. Ravin, and R. Y. Stanier. 1974. *Bergey's Manual of Determinative Bacteriology*. The Williams & Wilkins. Baltimore.
- Buncic, S., L. Paunovic, and D. Radisic. 1990. The fate of *Listeria monocytogenes* in fermented sausages and in vacuum-packaged frankfurters. *J. Food Prot.* 54:413-417.
- Buncic, S. 1991. The incidence of *Listeria monocytogenes* in slaughtered animals, in meat, and in meat products in Yugoslavia. *Intl. J. Food Microbiol.* 12:173-180.
- Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods- a review. *Intl. J. Food Microbiol.* 94:223-253.
- Center for Disease Control and Prevention. 2004. Diagnosis and management of food borne illness. *Morb. Mortal. Wkly Rep.* 53:1-33.
- Center for Disease Control and Prevention. 2003. Listeriosis, Technical information.  
[http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis\\_t.htm](http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis_t.htm)
- Center for Disease Control and Prevention. 2002. Multistate outbreak of listeriosis-United States. *Morb. Mortal. Wkly Rep.* 51:950-951.
- Center for Disease Control and Prevention. 2000. Multistate outbreak of listeriosis-United States. *Morb. Mortal. Wkly Rep.* 50:1129-1130.
- Center for Disease Control and Prevention. 2000. Preliminary foodnet data on the Incidence of foodborne Illnesses --- Selected Sites, United States. 2000. *Morb. Mortal. Wkly Rep.* 50:241-246.
- Center for Food Safety and Applied Nutrition (CFSAN). 2003. *Listeria* Risk Assessment.

<<http://www.foodsafety.gov/~dms/lmriskl.html>>(July, 2001).

Chen, N., and L. Shelef. 1991. Relationship between water activity, salt of lactic acid, and growth of *Listeria monocytogenes* in a meat model system. *J. Food Prot.* 55:574-578.

Choi, H. S., and K. B. Chin. 2003. Evaluation of sodium lactate as a replacement for conventional chemical preservative in comminuted sausages inoculated with *Listeria monocytogenes*. *Meat Sci.* 65:531-537.

Colburn, K. G., A. C. Kaysner, Jr. C. Abeyta, and M. M. Wekell. 1990. *Listeria* species in a California coast estuarine environment. *Appl. Environ. Microbiol.* 6: 53-156.

Deans, S. G., and G. Ritchie. 1987. Antibacterial properties of plant essential oils. *Intl. J. Food Microbiol.* 5:165-180.

Coronado, A. S., G. R. Trout, F. R. Dunshea, and N. P. Shah. 2002. Effect of dietary vitamin E, fishmeal and wood and liquid smoke on the oxidative stability of bacon during 16 weeks' frozen storage. *Meat Sci.* 62:51-60.

Cox, S. D., M. C. Mann, L. J. Markham, C. H. Bell, E. J. Gustafson, R. J. Warmington, and G. S. Wyllie. 2000. The mode of antimicrobial action of essential oil of *Melaleuca alternifolia* (tea tree oil). *J. Appl. Microbiol.* 88:170-175.

Daun, H. 1979. Interaction of wood smoke components and foods. *J. Food Technol.* 5:66-71, 83.

Deans, S. G., and G. Ritchie. 1987. Antibacterial properties of plant essential oils. *Intl. J. Food Microbiol.* 5:165-180.

De Wit, J. C., and F. M. Omboust. 1989. Antimicrobial activity of sodium lactate. *J. Food Microbiol.* 7:113-120.

Dillon, R., and T. Patel. 1993. Effect of cold smoking and storage temperatures on *Listeria monocytogenes* in inoculated cod fillets (*Gadus morhus*). *Food Research Intl.* 26:97-101.

Dillon, R., T. Patel, and S. Ratnam. 1994. Occurrence of *Listeria* in hot and cold smoked seafood products. *Intl. J. Food Microbiol.* 22:73-77.

Doyle, P. M. 1989. Foodborne bacterial pathogens. p. 283-298. Marcel Dekker. NY.

- Dykes, A. G. 2003. Behavior of *Listeria monocytogenes* on two processed meat products as influenced by temperature or attached growth during preincubation. *J. Food Microbiol.* 20:91-96.
- El-Shenawy, M. A., and H. E. Mart, 1988. Inhibition and inactivation of *Listeria monocytogenes* by sorbic acid. *J. Food Prot.* 51:842-847.
- El-Shenawy, M. A., and H. E. Mart, 1988. Sodium benzoate inhibits growth of or inactivates *Listeria monocytogenes*. *J. Food Prot.* 51:525-530.
- El-Shenawy, M. A., and H. E. Mart, 1989. Behavior of *Listeria monocytogenes* in the presence of sodium propionate. *Intl. J. Food Microbiol.* 8:85-94.
- Farber, J. M. 1990. Microbiological aspects of modified-atmosphere packaging technology- a review. *J. Food Prot.* 54:58-70.
- Farber, M. J., and B. E. Brown. 1990. Effect of prior heat shock on heat resistance of *Listeria monocytogenes* in meat. *Appl. Environ. Microbiol.* 56:584-1587.
- Farber, J. M., and I. P. Peterkin. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol. Rev.* 55:476-511.
- Farber, J. M. and E. Daley. 1994. Fate of *Listeria monocytogenes* on modified atmosphere packaged turkey roll slices. *J. Food Prot.* 57:1098-1100.
- Farber, J. M., Y. Cai, and W. H. Ross. 1996. Predictive modeling of the growth of *Listeria monocytogenes* in CO<sub>2</sub> environments. *Intl. J. Food Microbiol.* 32:133-144.
- Food and Drug Administration (FDA), U. S. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate, and potassium lactate. *Fed. Regist.* 65:3121-3123.
- FDA/Center for Food Safety and Applied Nutrition (CFSAN). 2001. Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Hazard Identification. 8-22.  
<http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/03-005N/buchanan.ppt>
- Food Safety and Inspection Service (FSIS). 2004. FSIS rules designated to reduce *Listeria monocytogenes* in ready-to-eat meat & poultry. *Fact sheets*.  
<http://www.fsis.usda.gov/Frame/FrameRedirect.asp?main=http://www.fsis.usda.gov/OA/background/lmfinal.htm>
- Food Safety and Inspection Service (FSIS). 1999. FSIS announces strategy to control *Listeria monocytogenes* in ready-to-eat meat and poultry products.

[http://www.fsis.usda.gov/OA/news/1999/lm\\_haccp.htm](http://www.fsis.usda.gov/OA/news/1999/lm_haccp.htm)

Food Safety and Inspection Service (FSIS). 2002. New jersey firm expands recall of poultry products for possible *Listeria* contamination.

<http://www.fsis.usda.gov/oa/recalls/prelease/pr098-2002.htm>

George, S. M., M. B. Lund, and F. T. Brocklehurst. 1988. The effect of pH and temperature on initiation of growth of *Listeria monocytogenes*. *J. Appl. Bacteriol.* 81:433-438.

Gill, A. O., P. Delaquis, P. Russo, and A. R. Holley. 2002. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Intl. J. Food Microbiol.* 73:83-92.

Gill, O. C., C.J. McGinnis, A. Houde, L. Lamoureux, D. Leblanc. 2005. Microbiological conditions of moisture-enhanced pork before and after cooking. *J. Food Microbiol.* 22:321-327

Glass, A. K., and Doyle. P. M. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl. Environ. Microbiol.* 55:1565-1569.

Gould, G. W. 1996. Methods for preservation and extension of shelf life. *Intl. J. Food Microbiol.* 33:51-54.

Gray, M. L. 1960. Isolation of *Listeria monocytogenes* from oat silage. *Science.* 132:1767-1768.

Gray, J. I., and A. M. Pearson. 1994. Lipid-derived off-flavors in meat formation and inhibition, p. 116-143. *In*. F. Shahidi (ed.), Flavor of meat and meat products. London: Chapman & Hall.

Grau, F. H., and B. P. Vanderlinde. 1991. Occurrence, number, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *J. Food Prot.* 55:4-7.

Guillen, M. D., and M. L. Ibargoitia. 1996. Relationships between the maximum temperatures reached in the some generation process from *Vitis vinifera* L. shoot sawdust and composition of the aqueous smoke flavoring preparation. *J. Agri. Food Chem.* 44:1302-1307.

Guillen, M. D., and M. L. Ibargoitia. 1998. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. *J. Agri. Food Chem.* 46:1276-1285

Giullen, M. D., P. Sopelana, and M. A. Partearroyo. 2000. Determination of ply aromatic hydrocarbons in commercial liquid smoke flavorings of different



compositions by gas chromatography-mass spectrometry. *J. Agri. Food Chem.* 48:126-131.

Giullen, M. D., and M. J. Manzanos. 2002. Study of the volatile composition of an aqueous oak smoke preparation. *J. Food Chem.* 79:283-292.

Guillen, M. D., N. Cabo. 2004. Study of the effect of smoke flavorings on the oxidative stability of the lipids of pork adipose tissue by means of fourier transform infrared spectroscopy. *Meat Sci.* 66:647-657.

Guyer, S., and T. Jemmi. 1991. Behavior of *Listeria monocytogenes* during fabrication and storage of experimentally contaminated smoked salmon. *Appl. Environ. Microbiol.* 57:1523-1527.

Harmayani, E., J. N. Sofos, and G. R. Schmidt. 1993. Fate of *Listeria monocytogenes* in raw and cooked ground beef with meat processing additives. *Intl. J. Food. Microbiol.* 18:223-232.

Hattula, T., K. Elfving, U. M. Mrouch, and T. Luoma. 2001. Use of liquid smoke flavorings as an alternative to traditional flue gas smoking of rainbow trout filets. *Lebensm-Wiss-U Technol.* 34:521-525.

Heisick, J. E., E. D. Wagner, L. M. Nierman, and T. J. Peeler. 1989. *Listeria* spp. Found on fresh market produce. *Appl. Environ. Microbiol.* 55:1925-1927.

Hu, A. C., and A. L. Shelef. 1996. Influence of fat content and preservatives on the behavior of *Listeria monocytogenes* in beaker sausage. *J. Food Safety.* 16:175-181.

Husu, J., T. J. Beery, E. Nurmi, and P. M. Doyle. 1990. Fate of *Listeria monocytogenes* in orally dosed chicks. *Intl. J. Food Microbiol.* 11:259-269.

James, M. J. 1996. Prevalence of *Listeria* spp. in meat and poultry products. *Food Control.* 7:209-214.

Johnson, J. L., M. P. Doyle, and R. G. Cassens. 1988. Survival of *Listeria monocytogenes* in ground beef. *Intl. J. Food Microbiol.* 6:243-247.

Johnson, J. M., J. Hirn, P. Hill, and E. Nurmi. 1989. Effect of different levels of nitrite and nitrate on the survival of *Listeria monocytogenes* during the manufacture of fermented sausages. *Intl. J. Food Microbiol.* 6:243-247.

Juneja, K. V., and J. N. Sofos. 2002. Control of foodborne microorganism. Marcel Dekker, NY.

- Junttila, J. M., J. Hill, and E. Nurmi. 1989. Effect of different levels of nitrite and nitrate on the survival of *Listeria monocytogenes* during the manufacture of fermented sausage. *J. Food Prot.* 52:158-161.
- Juven, B. J., S. F. Barefoot, M. D. Pierson, L. H. McCaskill, and B. Smith. 1997. Growth and survival of *Listeria monocytogenes* in vacuum-packaged ground beef inoculated with *Lactobacillus alimentarius* FlaoraCarn L-2. *J. Food Prot.* 61:551-556.
- Knabel, J. S., H. w. Walker, P. A. Hartman, and A. F. Mendonca. 1990. Effect of growth temperature and strictly anaerobic recovery on the survival of *Listeria monocytogenes* during pasteurization. *Appl. Environ. Microbiol.* 56:370-376.
- Konstantinos, P. K, P. A. Kendall, and J. N. Sofos. 2004. A comparative study on growth limits of *Listeria monocytogenes* as affected by temperature, pH and *a<sub>w</sub>* when grown in suspension or on a solid surface. *J. Food Microbiol.* 21:415–422
- Lanciotti, R., F. Patrignani, F. Bagnolini, M. E. Guerzoni, and F. Gardini. 2003. Evaluation of diacetyl antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. *J. Food Microbiol.* 20:537-543.
- Lemay, J. M., J. Choquette, P. J. Delaquis, C. Gariépy, N. Rodrigue, and L. Saucier. 2002. Antimicrobial effect of natural preservatives in a cooked and acidified chicken meat model. *Intl. J. Food Prot.* 78:217-226.
- Lamkey, J. W., W. F. Leak, B. W. Tuley, D. D. Johnson and L. R. West. 1991. Assessment of sodium lactate addition to fresh pork sausage. *J. Food Sci.* 56: 220-223.
- Lindquist, S. 1986. The heat-shock response. *Annu. Rev. Biochem.* 55:1151-1191.
- Liv, M. R. 2000. *Listeria monocytogenes* in the smoked salmon industry. *Intl. J. Food Microbiol.* 62:183-190.
- Lou, Y., and E. A. Yousef. 1999. Characteristic of *Listeria monocytogenes* important to food processors. In Ryser, E. T., and H. E. Marth. *Listeria, Listeriosis and food safety*, (ed.), Marcel Dekker, NY.
- Lis-Balchin, M., S. Hart, G. S. Deans, and E. Eaglesham. 1996. Comparison of the pharmacological and antimicrobial action of commercial plant essential oils. *J. Herbs, Spices and Medicinal Plants.* 4:69-86.
- Maas, R. M., K. A. Glass, and M. P. Doyle. 1989. Sodium lactate delays toxin production by *clostridium botulinum* in cook-in-bag turkey products. *Appl. Environ. Microbiol.* 55:2226-2229.

- Mackey, B. M., and N. Bratchell. 1989. The heat resistance of *Listeria monocytogenes*- a review. *Lett. Appl. Microbiol.* 9:89-94.
- Martinez, O., J. Salmeron, M. D. Guillen, and C. Casas, 2004. Texture profile analysis of meat products treated with commercial liquid smoke flavorings. *Food control.* 15:457-461.
- Mclauchlin, J. 1996. The relationship between *Listeria* and listeriosis. *Food Control.* 7:187-193.
- McClure, P. J., M. T. Kelly, and A. T. Robers. 1991. The effect of temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. *Intl. J. Food Microbiol.* 14:77-92.
- Meldrum, J. R., T. F. Brocklehurst, D. R. Wilson, and P. D. G. Wilson. 2003. The effect of cell immobilization, pH and sucrose on the growth of *L. monocytogenes* Scott A at 10°C. *J. Food Microbiol.* 20:97-103.
- Messina, M. C., A. H. Ahmad, A. J. Marchelo, P. C. Gerba, and W. M. Paquette. 1988. Effect of liquid smoke on *Listeria monocytogenes*. *J. Food Prot.* 51:629-631.
- Miller, R. K. and R. G. Acuff. 1994. Sodium lactate affects pathogens in cooked beef. *J. Food Sci.* 59:15-19.
- Miller, A. J., E. J. Call, and C. R. Whiting. 1992. Comparison of organic acid salts for *Clostridium botulinum* control in uncured turkey products. *J. Food Prot.* 56:958-962.
- Moura, M. S., M.T. Destro, and B. D. G. M. Franco. 1993. Incidence of *Listeria* species in raw and pasteurized milk produced in Sao Paulo, Brazil. *Intl. J. Food Microbiol.* 19:229-237.
- R.Y. Murphy, Y. R., B.L. Beard, E.M. Martin, A.E. Keener, and T. Osaili. 2004. Predicting process lethality of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* in ground, formulated, and formed beef/turkey links cooked in an air impingement oven. *J. Food Microbiol.* 21:493-499.
- Murray, E. G. D., R. A. Webb, and M. B. R. Swann. 1926. A disease of rabbits characterized by a large mononuclear leukocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). *J. Pathol. Bacteriol.* 29:407-439.

- National Advisory Committee on Microbiological Criteria for Foods (NACMCF). 1991. Foodborne listeriosis. *Int. J. Food Microbiol.* 14:185-246.
- Newberne, P., R. L. Smith, J. Doull, J. V. Feron, I. J. Goodman, C. I. Murno, S. P. Portuguese, J. W. Waddell, M. B. Wagner, S. C. Weil, B. T. Adams, and B. J. Hallagan. 2000. GRAS flavoring substances. *J. Food Technol.* 54:66-83.
- Niedziela, C. J., M. MacRae, I. D. Ogden, and P. Nesvadba. 1998. Control of *Listeria monocytogenes* in salmon; Antimicrobial effect of salting, smoking and specific smoke compounds. *Lebensm.-Wiss. U.-Technol.* 31:155-161.
- Palumbo, S. A., and A. C. Williams. 1993. Control of *Listeria monocytogenes* on the surface of frankfurters by acid treatments. *J. Food Microbiol.* 11:293-300.
- Papadopoulos, L. S., R. K. Miller, R. G. Acuff, C. Vanderzant, and R. H. Cross. 1991. Effect of sodium lactate on microbial and chemical composition of cooked beef during storage. *J. Food Sci.* 56:341-347.
- Pearson, A. M., J. I. Gray, A. M. Wolzak, and N. A. Horenstein. 1983. Safety implication of oxidized lipids in muscle foods. *J. Food Technol.* 37:121-129.
- Petran, R. L., and A. E. Zottala. 1989. A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *J. Food Sci.* 54:458-460.
- Pirie, J. H. H. 1940. *Listeria*: Change of a name for a genus of a bacterium. *Nature.* 145:264.
- Pszczola, D. E. 1995. Tour highlights production and uses of smokes-based flavors. *J. Food Technol.* 49:70-74.
- Razavilar, V., and C. Genigeorgis. 1998. Prediction of *Listeria* spp. growth as affected by various levels of chemicals, pH, temperature and storage time in a model broth. *Intl. J. Food Microbiol.* 40:149-157.
- Radecki, A., H. Lamparczyk, J. Grzybowski, and J. Halkiewicz. 1978. Separation of polycyclic hydrocarbons and determination of benzo-pyrene in liquid smoke preparation. *J. Chromatography.* 150:527-532.
- (NACMCF) Recommendations by the National Advisory Committee on Microbiological Criteria for Foods. 1991. *Listeria monocytogenes*. *Intl. J. Food Microbiol.* 14:185-246.
- Rhodehamel, E. J., 1992. FDA's concerns with sous vide processing. *J. Food Technol.* 46:73-76.

- Rijpens, N. P., G. Jannes, and F. M. L. Herman. 1996. Incidence of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat chicken and turkey products determined by polymerase chain reaction and line probe assay hybridization. *J. Food Prot.* 60:548-550.
- Rongrong, L., J. A. Carpenter, and R. Cheney, 1998. Sensory and instrumental properties of smoked sausage made with technically separated poultry (MSP) meat and wheat protein. *J. Food Sci.* 63:923-929.
- Rorvik, M. L. 2000. *Listeria monocytogenes* in the smoked salmon industry. *Intl. J. Food Microbiol.* 62:183-190.
- Ryser, E. T., and E. H. Marth. 1991. *Listeria monocytogenes* characteristic and classification. *Listeria, listeriosis and food safety*, Marcel Dekker. NY.
- Sallam, I. Kh., and K. Samejima. 2004. Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. *Lebensm.-Wiss. u.-Technol.* 37:865–871
- Sanna, M., B. Poutrel, J. L. Menard, and F. Serieys. 1993. Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. *J. Dairy Sci.* 76:2891-2898.
- Shahidi, F. 1994. Assessment of lipid oxidation and off-flavor development in meat and meat products, p. 247-266. *In* F. Shahidi (ed.), *Flavor of meat and meat products*. Chapman and Hall, London.
- Salih, M. A., E. W. Sandine, and J. A. Ayres. 1990. Inhibitory effect of Microgard on yogurt and cottage cheese spoilage organisms. *J. Dairy Sci.* 73:887-889.
- Samelis, J., G. K. Bedie, N. J. Sofos, E. K. Belk, A.J. Scanga, and C. G. Smith. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* post-processing contamination on frankfurters stored at 4 degree C in vacuum packages. *J. Food Prot.* 64:1949–1955.
- Schlech, F W. 1996. Overview of listeriosis. *Food Control.* 7:181-186.
- Schlech, F. W., M. P. Lavigne, and A. R. Bortolussi, C. A. Allen, V. E. Haldane, A. J. Wort, E. S. Johnson, H. S. King, S. E. Nicholls, and V. C. Broome. 1983. Epidemic listeriosis: evidence for transmission by food. *N. Engl. J. Med.* 308:203-206.
- Schlyter, J. H., K. A. Glass, J. Loeffelhol, A. J. Degnan and J. B. Luchansky. 1993. The effects of diacetate with nitrite, lactate, or pediocin on the viability of *Listeria monocytogenes* in turkey slurries. *Intl. J. Food Microbiol.* 19:271-281

Seeliger, H. P. R., and D. Jones. 1987. *Listeria*, p. 1235-1245. In Bergey's Manual of Systematic Bacteriology, (ed.), The Williams and Wilkins. Baltimore.

Seeliger, H. P. R., and D. Jones. 1986. *Listeria*, p. 1235-1245 In P. H. A. Sneath, S. N. Mair, E. M. Sharpe, and G. J. Holt (ed.). Bergey's Manual of Systematic Bacteriology. The Williams & Wilkins. Baltimore.

Seeliger, H. P. R., and D. Jones. 1993. *Listeria*. P. 1235-1245. In. J. G. Holt (ed.), Bergey's Manual of Systematic Bacteriology. The William & Wilkins. Baltimore.

Shelef, L. A., and L. Addala. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J. Food Safety*. 14:103-115.

Shelef, L. A. 1983. Antimicrobial effects of spices. *J. Food Safety*. 6:29-44.

Simko, P. 2002. Determination of polycyclic aromatic hydrocarbons in smoked meat products and smoke flavoring food additives. *J. Chromatography B*. 770:3-18.

Singh, R.K. Singh, A.K. Bhunia, and N. Singh. 2003. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. *Lebensm.-Wiss. u.-Technol*. 36:787-794

Sink, D. J., and L. A. Hsu. 1978. Chemical effects of smoke processing on frankfurter quality and palatability characteristics. *Meat Sci*. 3:247-253.

Skovgaard, N., and A. C. Morgen. 1988. Detection of *Listeria* spp. In faeces from animal, in feeds, and in raw foods of animal origin. *Intl. J. Food Microbiol*. 6:229-242.

Smith-palmer, A., J. Stewart, and L. Fyle. 2001. The potential application of plant essential oils as natural food preservatives in soft cheese. *J. Food Microbiol*. 18:463-470.

Stekelenburg, K. F. 2003. Enhanced inhibition of *Listeria monocytogenes* in frankfurters sausage by the addition of potassium lactate and sodium diacetate mixtures. *J. Food Microbiol*. 20:133-137.

Susen, E., 1998. Minimum inhibitory concentration of smoke wood extracts against spoilage and pathogenic micro-organisms associated with foods. *Lett. Appl. Microbiol*. 27:45-48.

Schwanke, S., W.G. Ikins, C. Kastner and M.S. Brewer. 1996. Effects of liquid smoke on lipid oxidation in a beef model system and restructured roasts. *J. Food Lipids* 3:99-113.

Tassou, C., H. E. Drosinos, and E. J. G. Nychas, 1995. Effects of essential oil from mint (*Mentha piperita*) on *Salmonella enteritidis* and *Listeria monocytogenes* in model food systems at 4°C and 10°C. *J. Appl. Bacteriol.* 78:593-600.

Tham, W., H. Ericsson, S. Loncarevic, H. Unnerstad, and M. L. Danielsson-tham. 2000. Lessons from an outbreak of listeriosis related to vacuum-packed gravid and cold-smoke fish. *Intl. J. Food Microbiol.* 62:173-175.

Thevenot, D., M. L. D. Muller, S. Christieans, and C. V. Rozand. 2005. Prevalence of *Listeria monocytogenes* in 13 dried sausage processing plants and their products. *Intl. J. Food Microbiol.* Article in Press.

Toth, L., and K. Potthast. 1984. Chemical aspects of the smoking meat and meat products. *Adv. Food Res.* 29:87-158.

Thurette, J., J. M. Membre, L. H. Ching, R. Tailliez, and M. Catteau. 1998. Behavior of *Listeria spp.* in smoked fish products affected by liquid smoke, NaCl concentration, and temperature. *J. Food Prot.* 61:1475-1479.

Van den Elzen, A. M. G., and M. A. J. Snijders. 1993. Critical points in meat production lines regarding the introduction of *Listeria monocytogenes*. *Vet. Quart.* 15:143-145.

Vitas, I. A., V. Aguado e, and I. Garcia-Jalon. 2004. Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain). *Intl. J. Food Microbiol.* 90:349-356.

Wang, C., and P. M. Muriana. 1993. Incidence of *Listeria monocytogenes* in packages of retail franks. *J. Food Prot.* 57:382-386.

Wederquist, H. J., J. N. Sofos, and R. G. Schmidt. 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. *J. Food Sci.* 59:498-500.

Wendorff, W. L., W. E. Riha, and E. muehlenkamp. 1993. Growth of molds on cheese treated with heat or liquid smoke. *J. Food Prot.* 56:963-966.

WHO, 2002. Food safety and foodborne illness. World Health Organization fact sheet 237. Geneva.

<http://www.who.int/mediacentre/factsheets/fs237/en/>

WHO, 1988. Foodborne listeriosis. World Health Organization. Document no. WHO/WHE/FOS/88.S, Geneva.

<http://www.journals.uchicago.edu/CID/journal/issues/v31n3/000554/000554.html>

Yucel, N., S. Citak, and M. Onder. 2005. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. *J. Food Microbiol.* 22:241-245.

Zaika, L. L., A. S. Palumbo, L. J. Smith, F.C. Del, S. Bhaduri, O. C. Jones, and H. A. Kim. 1989. Destruction of *Listeria monocytogenes* during frankfurters processing. *J. Food Prot.* 53:18-21.



## CHAPTER II

### Effect of liquid smoke extracts on the shelf life of frankfurters inoculated with *Listeria monocytogenes*

#### 2. 1. Abstract

*Listeria monocytogenes* has been troublesome for many ready-to-eat (RTE) meats, including hotdogs, which have had among the highest incidence of *Listeria* contamination among fully-cooked RTE meat products. Our objectives were to examine the effect of liquid smoke control of *L. monocytogenes* on hotdogs. Hotdogs were either obtained locally at retail (containing lactate/diacetate) or manufactured for us by a local processor without added lactate/diacetate. In challenge studies of retail franks containing lactate/diacetate, low levels of *L. monocytogenes* were able to increase to significantly higher levels on 5 of 8 brands tested when held at 1.6°C (35°F). Liquid smoke was able to reduce and control growth of *L. monocytogenes* on franks when treated for as long as 90 sec and as short as 5 sec versus untreated controls. Effective control of *L. monocytogenes* was also obtained for 10 weeks when dipped for as short as 1 sec or when dropped through an atomized mist produced by a pressurized spray canister. Hotdogs were also manufactured without lactate/diacetate by a

large commercial manufacturer of franks and sprayed with liquid smoke using a commercial device as they exited the peeler. When inoculated at 3 different levels ( $10^1$ ,  $10^2$ ,  $10^3$  CFU) with a 4-strain cocktail of *L. monocytogenes* and stored at abuse temperatures of 6°C (43°F), the smoke-treated samples again demonstrated effective control of *L. monocytogenes* relative to untreated control samples. The data shows that surface application of liquid smoke extracts by dipping or spraying may inhibit the growth of *L. monocytogenes* on hotdogs during shelf life and should facilitate a claim as an Alternative 2, and possibly Alternative 1, process for HACCP purposes.

Key Words: *L. monocytogenes*, liquid smoke, frankfurters, hotdogs, HACCP.

## 2. 2. Introduction

*L. monocytogenes* is a foodborne pathogen that causes listeriosis in humans as well as animals (Siegman-Igra et al., 2002). It is a rod-shaped, Gram-positive, non-sporeforming, facultatively anaerobic bacterium (Farber and Peterkin, 1991). *L. monocytogenes* can enter host cells and spread both intra- and inter-cellularly, resulting in the spread of infection. Groups that are particularly at risk are the elderly, the immuno-compromised, pregnant women and their unborn fetuses.

*L. monocytogenes* is widely distributed in nature and has been found on decaying vegetation, in soils, animal feces, sewage, silage, water, as well as in food processing environments (Jay, 2000). It has the ability to grow on most non-acid foods. These characteristics offer *L. monocytogenes* plenty of opportunity to enter the food chain and multiply (Adam and Moss, 2002). *L. monocytogenes* can grow over a wide range of temperatures, and even at refrigeration temperature, especially if abused. It is more heat-resistant than other gastrointestinal foodborne pathogens (i.e., *Salmonella*, *E. coli* O157:H7) and can survive freezing and drying. In the food industry, a major concern has been transmitting the organism through ready-to-eat (RTE) meat products whereby consumers are not expected to cook the food prior to consumption.

According to the Centers for Disease Control and Prevention (CDC), 76 million illnesses, 325,000 hospitalizations, and 5000 deaths occur annually in the U.S. due to foodborne diseases. Of that, *L. monocytogenes* is estimated to be responsible for 2500 illnesses and 500 deaths each year (Murphy et al., 2005). In a recent outbreak, seven deaths and three stillbirths occurred due to the

consumption of *L. monocytogenes*-contaminated sliced turkey deli meat products in the northeast United States resulting in the recall of 27.4 million lbs of fresh and frozen RTE poultry products (CDC, 2002).

The significance of listeriosis demonstrates that there is a need for controlling *Listeria* in food processing plants especially in those producing RTE products. According to USDA-FSIS Directive 10,240.3, products may be placed in high/medium or low-risk product categories (Anonymous, 2002). Based on this directive, products could be changed from high-risk to low-risk if using a postprocess lethality step or antimicrobials to control *Listeria*. The USDA-FSIS Final Rule identified three process category alternatives for manufacturers of RTE meat products (Anonymous, 2003a). An Alternative 3 process controlled *L. monocytogenes* by sanitation alone and thereby had the highest level of FSIS product testing. An Alternative 2 process had either a postprocess lethality step or antimicrobials in addition to sanitation, and therefore had less product testing than an Alternative 3 process. An Alternative 1 process included *both* a postprocess lethality step and ingredients to control *L. monocytogenes* during shelf life in addition to sanitation and therefore, received the least product testing. If an ingredient provides for both reduction of *L. monocytogenes* and control of growth during shelf life, it is possible for its use alone to achieve Alternative 1 status. Due to the incentives provided by the recent directives and final rule (i.e., less FSIS testing), efforts are currently being made by industry to identify new and emerging processes and antimicrobials that are effective in suppressing or eliminating *L. monocytogenes* on processed meat products.

Traditional smoking of meat and meat products imparts the smokey wood flavor to meat by longterm heating in the presence of burning wood chips. The advent of liquid smoke condensates has hastened the development of smoke flavor on treated meat products and shortened the overall processing time. Although the main functions of liquid smoke are to develop aroma, flavor, and color, recent work indicates additional attributes such as antioxidant properties and inhibition of microorganisms, especially *L. monocytogenes* (Messina et al., 1988; Sunen et al., 2003; Coronado et al., 2002). The main advantages of liquid smoke are that it can be applied quickly and inexpensively. The purpose of this study was to test the efficacy of a commercial liquid smoke condensate as an antimicrobial against *L. monocytogenes* on frankfurters for the purpose of achieving a lower risk product category.

## **2. 3. Materials and Methods**

**2. 3. 1. Bacterial strains.** The four strains of *L. monocytogenes* used in this study were Scott A-2 (serotype 4b; clinical isolate), V7-2 (serotype 1/2a; milk isolate), 39-2 (retail hotdog isolate), and 383-2 (ground beef isolate). Cultures were transferred twice from frozen stocks stored at -75°C into individual brain heart infusion (BHI) broth tubes at 1% inoculum level, and incubated overnight at 30°C. Overnight cultures were then mixed in equal proportions and 1 ml of mixed culture was used for making dilutions in 0.1% buffered peptone water (BPW) to obtain specific inoculum levels for various trials. These strains were resistant to both streptomycin (100 µg/ml; Sigma Chemical Co., St. Louis, MO) and rifamycin S/V (10 µg/ml; Sigma) and plating was performed on tryptic soy agar (TSA; Difco, Becton-Dickenson, Franklin Lakes, NJ) containing these antibiotics to exclude the detection of indigenous contaminating bacteria from non-sterile food products.

**2. 3. 2. Smoke extracts.** A commercial supplier of liquid smoke condensates (Mastertaste, Inc., Monterey, TN) provided the liquid smoke extract (Zesti-B™) used in this study. Zesti-B™ is Zesti-AM2 (cat no. 1042004) that has been adjusted to pH 4.2-4.4, contains 3.5-5.6% titratable acidity, and has a specific gravity of 1.1-1.2 gm/ml. The smoke extract used in this study is a refined fraction; these products have low-to-no smoke flavor based on the product selected. Products treated with liquid smoke extracts are generally listed as

“smoked” on ingredient labels (Anonymous, 2003b). The extract was stored at room temperature and used directly at full strength as received.

**2. 3. 3. Sample preparation and treatments.** Frankfurters used in this study were either purchased locally and contained lactate and/or diacetate, or were manufactured for us without lactate/diacetate. For our retail challenge study, retail franks from the same product/brand, and of the same use-by date, were commingled and rinsed briefly with a liter of chilled sterile water, then bagged, inoculated, vacuum-packaged, and stored for periodic shelf life testing.

**2. 3. 4. Smoke treatments (Fig. 2).** The hotdog brand that allowed high-level growth of *L. monocytogenes* in the retail challenge study was used for testing various dip times with liquid smoke extract. Franks were rinsed with sterile water and held briefly in a refrigerator before treatment with liquid smoke. In one study, hotdogs were dipped for 0 (control), 5, 15, 30, 60, and 90 sec, and placed two per bag into vacuum packaging bags. In another study, hotdogs were dipped for 0 (control), 1 sec, 5 sec, or applied by spray with a pressurized spray canister in which franks were dropped through a continuous mist of liquid smoke (Fig. 1A). In both studies, bagged samples were inoculated with  $\sim 10^1$  CFU of *L. monocytogenes*, vacuum-packaged, stored at 1.7°C (35°F) for 10 weeks and plated weekly.

Franks that were dipped in liquid smoke were allowed to drip dry for 5 min in the refrigerator to allow excess liquid smoke to run off to standardize the

amount of free liquid entering the packaging bags. Untreated controls were dipped in sterile water and held for a similar 'drip' period before bagging. After bagging (2 per bag), 0.5-1.0 ml of inoculum was added, massaged to distribute, and then vacuum-sealed and placed in temperature-controlled refrigerated storage at 1.7°C (35°F) for shelf life testing at various weekly intervals.

**2. 3. 5. Hotdogs manufactured without lactate and diacetate, sprayed with liquid smoke, and challenged with different levels of *L. monocytogenes*.** In another study, the effect of liquid smoke against different levels of *L. monocytogenes* was tested. Hotdogs were manufactured for this study without lactate and diacetate and sprayed with liquid smoke extract using a commercial spraying device as they exited the peeler (Fig. 1b). The manufactured franks were packaged in retail packages for transfer to our facility where they were stored frozen until used. Upon use, frankfurter samples were transferred to new vacuum bags to which 0.5-ml of a 4-strain mixture of *L. monocytogenes* was added at one of three different inoculation levels ( $10^1$ ,  $10^2$ , or  $10^3$  CFU). Purge from smoke-treated packages was also distributed to the new packages in order to minimize loss of smoke extract. Controls were also done at each inoculation level without liquid smoke treatment. Sample bags were vacuum-sealed and stored at 6.1°C (43°F) for ten weeks to simulate abuse temperature conditions. Treatments inoculated with  $10^1$  CFU were plated weekly while the other inoculation levels were sampled biweekly. Each treatment was performed in triplicate replications.



**2. 3. 6. Microbiological analysis.** Before plating, 2-ml of 0.1% BPW was added to opened sample packages which were massaged for a few minutes to resuspend surface bacteria into the rinse buffer which was then plated. Recovery of the rinse buffer was followed by appropriate serial dilutions and either pour-plated or spiral-plated depending on the level of cells expected. Spiral plating was done using the Eddy Jet (IUL Instruments, Cincinnati, OH). Plates were then incubated for 48 hrs at 30°C (86°F). Colony counts were obtained using an automatic colony counter (IUL Counterflash 4.2, IUL Instruments).

**2. 3. 7. Statistical analysis.** All trials were performed in triplicate replications. For most studies different replications were done on separate days with different lots of the same product. The collected data from three replicates were subjected to one-way repeated measures analysis of variance (RM ANOVA) to determine the differences between the different liquid smoke treatments in each period using Sigma Stat 3.1 (Systat Software, Inc., Richmond, USA). All pair-wise multiple comparisons were done using the Holm-Sidak method.

## 2. 4. Results and Discussion

When we challenged retail hotdogs that contained lactate and diacetate inoculated with a low level of *L. monocytogenes* (i.e.,  $\sim 10^1$  CFU) and incubated them at 1.7°C (35°F), *L. monocytogenes* rose to significantly higher levels on 5 of 8 brands tested, indicating ineffective inhibition of *Listeria* and demonstrating the need for more effective control on such products (Fig. 3). Currently, potassium lactate and sodium diacetate are the 'industry standard' in chemical preservatives that are widely used by the processed meat industry, and especially hotdogs. The use of these preservatives allows processors to attain Alternative II process category status (or Alternative I if it possesses both lethality and growth inhibition). Although levels of lactate/diacetate in the various brands weren't quantified by analytical testing, the main point remains that even low levels of added *Listeria* increased to higher levels under good refrigeration conditions.

The possible reasons for variation in lactate/diacetate between different brands of hotdogs that could affect different microbial response are, a) difference in functional effects due to hotdog formulation, b) difference in levels used by the various manufacturers (i. e) use of less lactate, c) use of less lactate/diacetate because it affects on flavor. Another explanation could be the brief rinse and repackaging in our protocol which could allow for diffusive loss of lactate/diacetate at the surface of the hotdogs. However, it is clear that under these same handling conditions, *Listeria* still did not increase significantly on 3 of 8 brands tested (data not shown).

For these reasons, we examined the use of liquid smoke extracts on one of the brands that was most permissive for growth of *L. monocytogenes* in our retail challenge study (i.e., Brand A). The liquid smoke extract was effective in preventing growth of *L. monocytogenes* when dipped for 90, 60, 30, or 15 sec in the liquid smoke fraction, and even with as short a dip time as 5 sec whereas, prolific growth of *L. monocytogenes* occurred on untreated controls (Fig. 4A). No significant differences were observed between treatments dipped for 5, 15, 30, 60, and 90 secs. However, a significant difference ( $P<0.05$ ) was observed between untreated controls and all smoke treatments in which viable counts were reduced below detection limits (Fig. 4A).

After these initial results, we were interested in examining even shorter treatment times that could be easily applied in the industry. Since a 5 sec dip time was effective reducing and preventing outgrowth of *L. monocytogenes*, we proceeded to examine the shortest possible treatment times of 1 sec and spray treatments. Effective control of *L. monocytogenes* was observed for as long as 10 weeks when using either a 1 sec dip time or a simple spray device to deliver the active agent compared to untreated controls (Fig. 4B). Significant differences ( $P<0.05$ ) were observed between controls versus dip or spray treatments but not between dip and spray treatments. Hence, reduction or control of the growth of *L. monocytogenes* has been achieved either by dipping or spraying the retail hotdogs with liquid smoke that would otherwise allow prodigious growth of *L. monocytogenes*.

The effect of liquid smoke was next examined on commercial franks manufactured without lactate and diacetate in which the smoke was applied by using a commercial spray applicator that would spray hotdogs as they were shot out through the peeler apparatus (Fig. 1B). The commercial sprayer uses a conical recovery section that catches and re-circulates unused liquid smoke. In this study, trials were performed at three different levels of inoculation, each in triplicate replications, and using a higher shelf life temperature of 6.1°C (43°F) representing abuse storage conditions. However, because of limitations with commercial processor downtime, all hotdogs were manufactured during one shift and used for the 3 replications. The results showed that with hotdogs inoculated at  $\sim 10^1$  CFU, *L. monocytogenes* declined quickly to undetectable levels and remained that way for the entire test period (Fig. 5A). At  $10^2$  CFU inoculation level, the levels slowly declined over a 4 week period and at  $10^3$  CFU inoculum level, *Listeria* slowly increased over 10 weeks to only 0.8 log higher than initial (high) inoculation levels whereas untreated controls increased by nearly 7 logs (Fig. 5B & 4C).

Although this study still demonstrated control of *L. monocytogenes* with liquid smoke extracts at abuse temperatures, further investigation of several conditions under which this trial was run could render even better results. The level of liquid smoke applied to the franks by the commercial sprayer may have been less than the treatment received from our manual spray applicator. The commercial peeler ejects franks at a much faster rate through the commercially sprayed liquid smoke 'curtain' than the rate at which we manually dropped franks through our

pressurized bottle spray mist (Fig. 1). This could be improved by having franks ejected through a longer orifice, requiring passage through several curtains of liquid smoke. Also, the hotdogs for this last trial series were manufactured, sprayed, and vacuum-packaged at the manufacturer's facilities from which we would later open the packages (smoke-treated and untreated controls) and repackage them with the challenge inoculum at our facilities. The hotdogs used in Fig. 5 were kept frozen prior to use, indicating that the effectiveness of the inhibitory properties of the liquid smoke remained intact over time as you might expect some adsorptive dilution of its concentration at the hotdog surface. We would also expect that due to some loss of the antimicrobial during transfer from the original packaging by re-packaging during the challenge inoculation, as well as loss due to adsorptive dilution at the surface, we feel we may have had even better results had we used them quickly after manufacture and/or re-inoculated them in their original packaging. However the results still demonstrate effective control at higher levels of *L. monocytogenes* than would be expected from typical contact contamination.

Hotdogs are one RTE product category that currently employs widespread use of inhibitors throughout the volume mass of product for a problem that is largely restricted to the surface via potential contact contamination. We have examined the use of surface application of liquid smoke extract to reduce and inhibit the growth of *L. monocytogenes* contamination on RTE frankfurter products and feel that the data shows there is merit in consideration of these

types of inhibitors towards reducing risk of *L. monocytogenes* on RTE meat products.

## **2. 5. Acknowledgements**

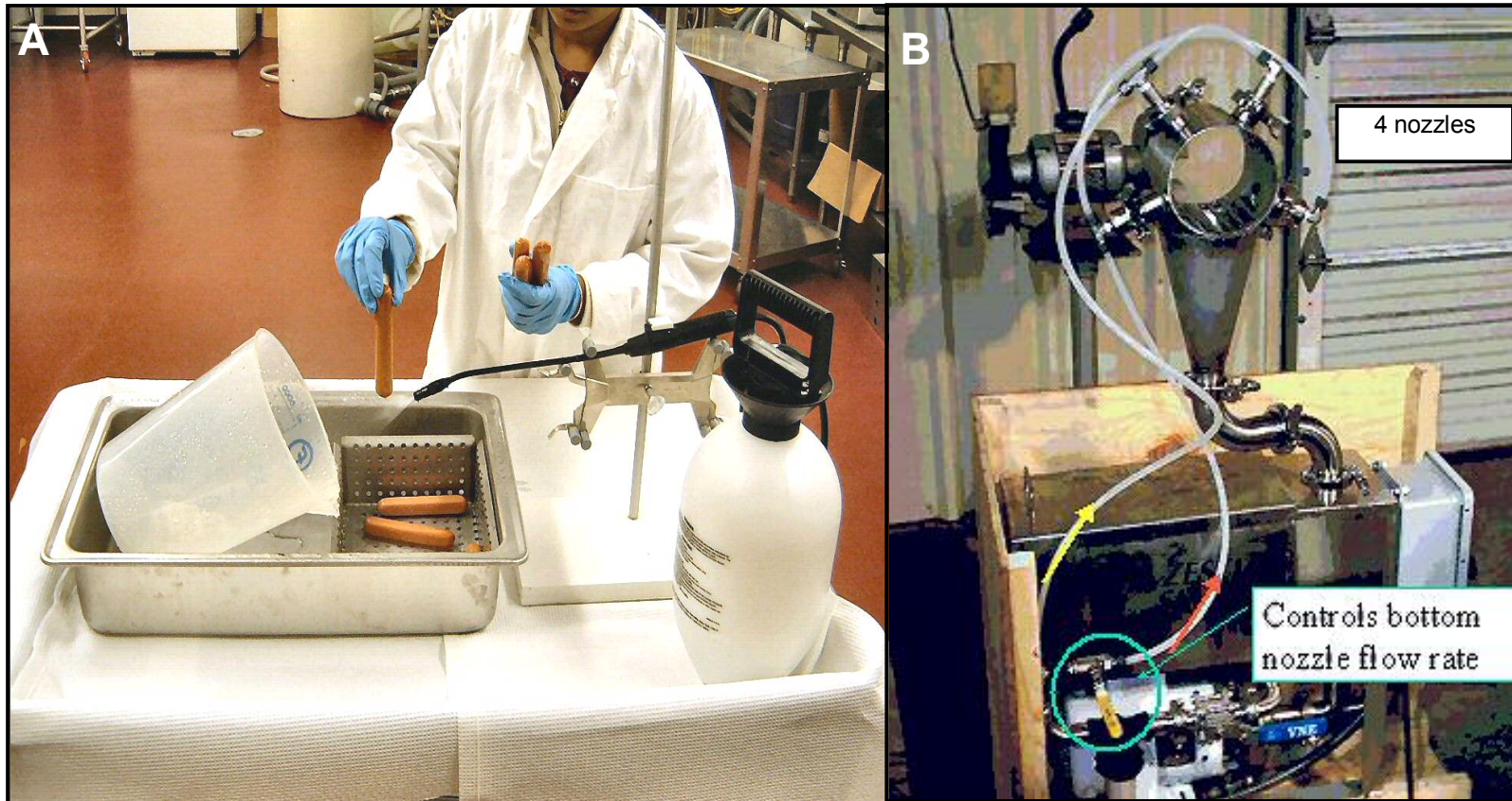
This research was partially funded by the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK. I would like to thank Mastertaste Inc. and Bar-S Foods Inc. for providing product, technical, and/or financial assistance during the course of this project.

## 2. 6. References

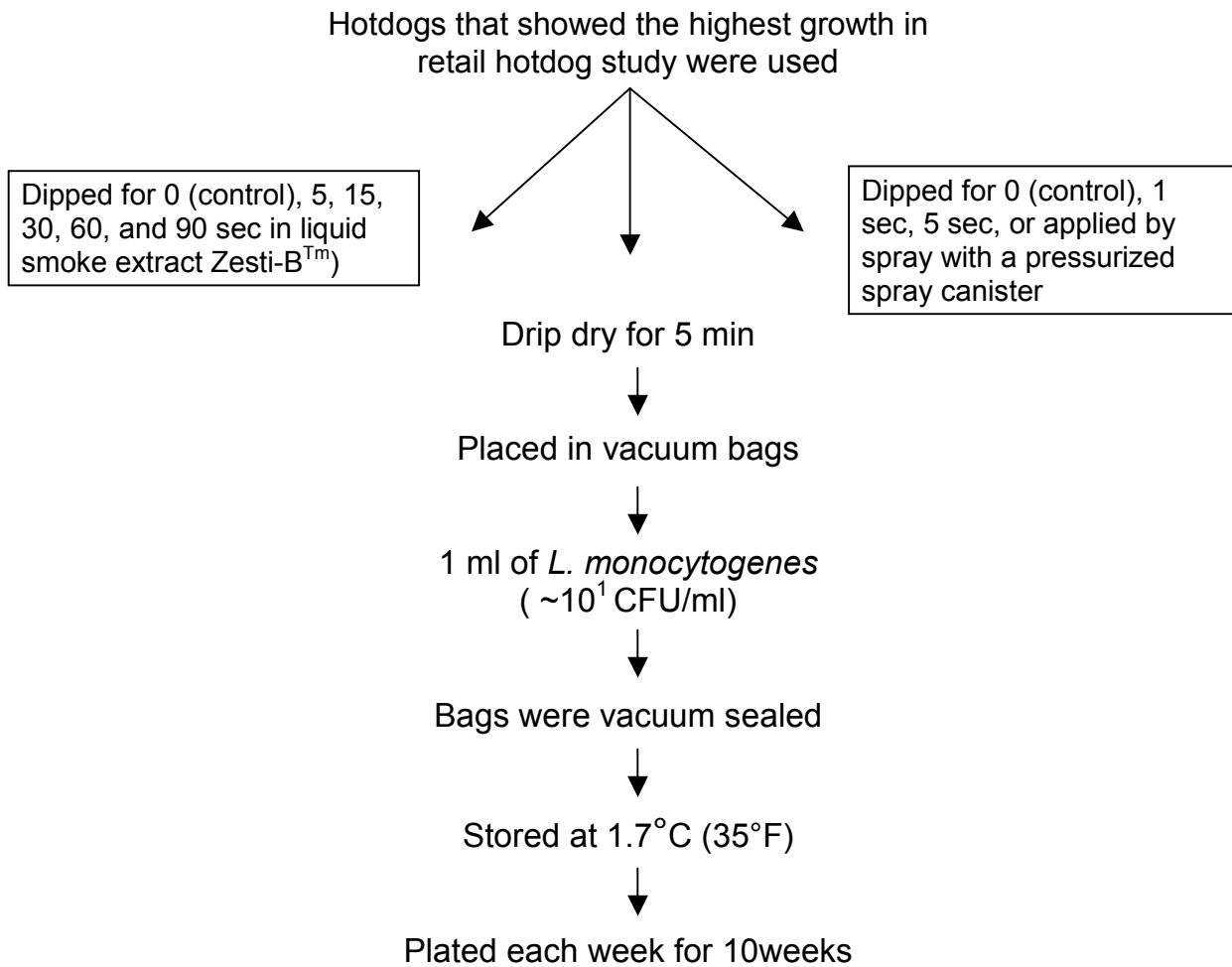
- Adams, R. M., and M. O. Moss. 2000. Food microbiology. University of Surrey, Guildford, UK.
- Anonymous. 2002. Microbial sampling of Ready-to-Eat products for the FSIS verification testing program. USDA-FSIS Directive 10,240.3.
- Anonymous. 2003a. Control of *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products; Final Rule. Fed. Reg. 68:34207-34254.
- Anonymous. 2003b. Food Standards and Labeling Policy Book. USDA-FSIS, Office of policy program development (May, 2003), p.152.
- Center for Disease Control and prevention. 2002. Multistate outbreak of listeriosis-United States. Morb. Mortal. Wkly Rep. 51:950-951.
- Coronado, A. S., G. R. Trout, F. R. Dunshea, and N. P. Shah. 2002. Effect of dietary vitamin E, fishmeal, wood and liquid smoke on the oxidative stability of bacon during 16 weeks' frozen storage. *Meat Sci.* 62:51-60.
- Farber, J. M., and I. P. Peterkin. 1991. *Listeria monocytogenes*, a foodborne pathogen. *Microbiol. Rev.* 55:476-511.
- Jay, M. J (ed.). 2000. Modern Food Microbiology. Aspen publishers, Inc. Gaithersburg, MD.
- Messina, M.C., H.A. Ahmad, J.A. Marchello, C.P. Gerba, and M.W. Paquette. 1988. The effect of liquid smoke on *Listeria monocytogenes*. *J. Food Prot.* 51:629-631.
- Murphy, Y. R., R. E. Hanson, L. K. Duncan, N. Feze, and B. G. Lyon. 2005. Considerations for post-lethality treatments to reduce *Listeria monocytogenes* from fully cooked bologna using ambient and pressurized steam. *Food Microbiol.* 22:359-365.
- Siegman-Igra, Y., R. Levin, M. Weinberger, Y. Golan, D. Schwartz, Z. Samana, H. Kongsberger, A. Yinnon, G. Rahav, N. Keller, N. Bishrat, J. Karpuch, R. Finkelstein, M. Alkan, Z. Shimoni, R. Lang, and T. Shohat. 2002. *Listeria monocytogenes* infection in Israel and review of cases worldwide. *Emerging infectious diseases.* 8:305-310.
- Singh, R., K. Singh, A.K. Bhunia, and N. Singh. 2003. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. *Lebensm.-Wiss. u.-Technol.* 36:787-794.

Sunen, E., C. Aristimuno, and B. Fernandez-Galian. 2003. Activity of smoke wood condensates against *Aeromonas hydrophila* and *Listeria monocytogenes* in vacuum-packaged, cold-smoked rainbow trout stored at 4°C. *Food Res. Intl.* 36:111-116.

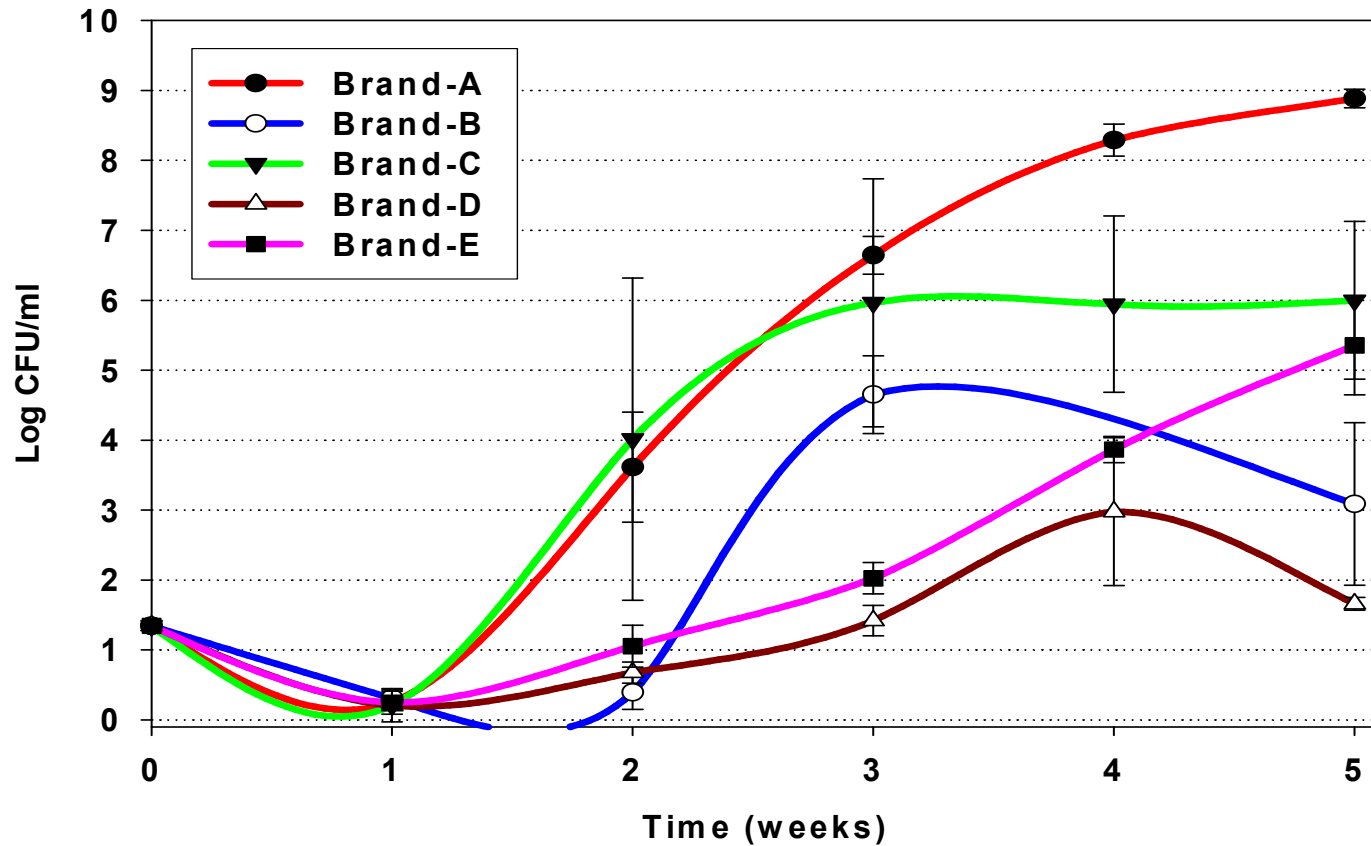




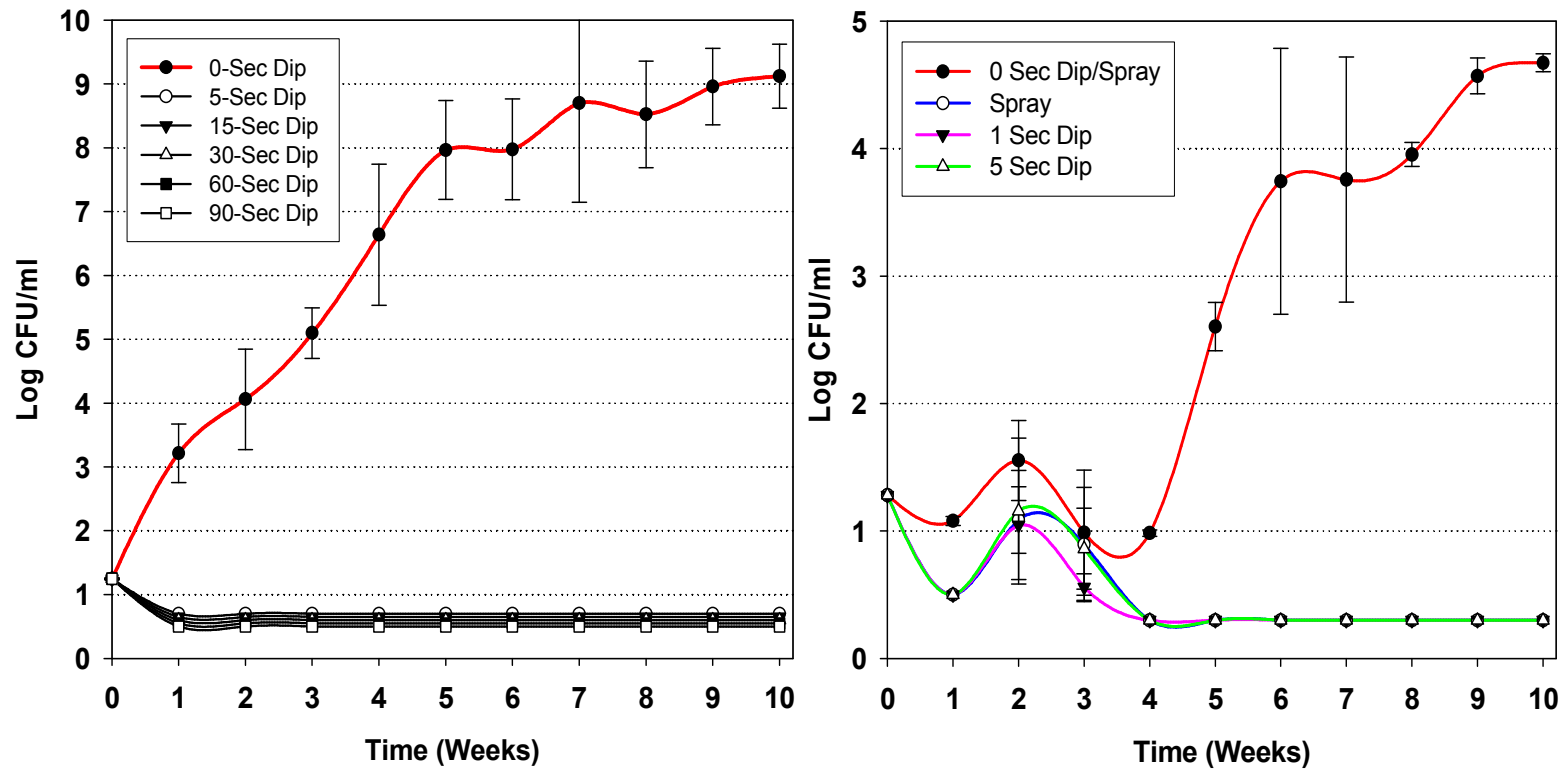
**Figure 1.** Spray applicators used in this study. Panel A, home-made setup for spraying liquid smoke extracts onto samples in our laboratory. Hotdogs were dropped through the spray mist after locking the handle in the spray position. Panel B, commercial device with 4 nozzles and pump mechanism to re-circulate unused solution. The device was attached to the outside of a peeler unit and hotdogs were sprayed as they were ejected through the opening.



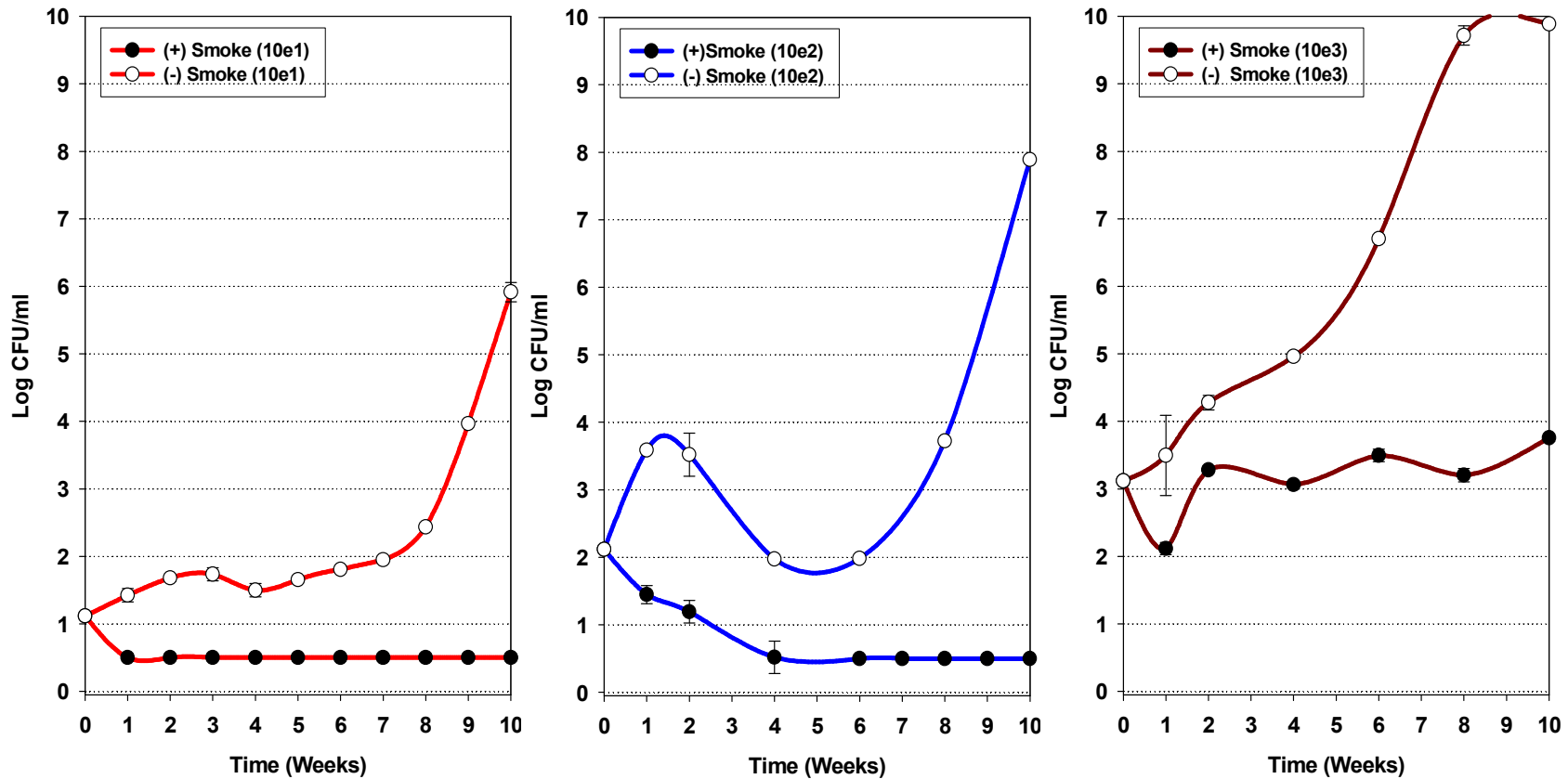
**Figure 2.** The flow chart of different dipping times as well as the spray against the growth of *L. monocytogenes* on hotdogs with low level inoculum



**Figure 3.** Inoculation of 5 retail brands of hotdogs that contain lactate/diacetate with a low-level inoculum mixture of 4 strains of *L. monocytogenes*, incubated at 1.7°C (35°F), and tested weekly for *L. monocytogenes*. The data represents the means of six replications. Similar testing with 3 additional brands did not show appreciable growth of *L. monocytogenes* during the test period.



**Figure 4.** Retail hotdogs shown to allow prodigious growth of *L. monocytogenes* (Brand A) were used to test various treatment times with Zesti-B™ liquid smoke extract. Panel A, hotdogs dipped for 0 (untreated), 5, 15, 30, 60, and 90 sec in liquid smoke extract, allowed to drip dry, and inoculated with a low level of a 4-strain mixture of *L. monocytogenes*. Panel B, same brand of retail hotdogs used in the prior study were again used to examine shorter treatment time and/or spray application of the liquid smoke extract using a pressurized spray canister. All treatments were done in triplicate replications. Samples were thoroughly massaged before vacuum packaging to facilitate inoculum distribution and then stored at 1.7°C (35°F) for 10 weeks. Error bars represent standard deviation of the mean.



**Figure 5.** Treatment of retail hotdogs with liquid smoke extract. Hotdogs were manufactured for this study without lactate and diacetate and sprayed with liquid smoke extract as they exited the peeler using a commercial spraying device. Untreated controls and smoke-treated samples were inoculated with 3 different levels of a 4-strain mixture of *L. monocytogenes* and held at 6°C (43°F) abuse conditions for 10 weeks. Data points represent the mean of triplicate replications

## CHAPTER III

### Effect of liquid smoke extracts in combination with heating on the shelf life of RTE meats inoculated with *Listeria monocytogenes*

#### 3. 1. Abstract

Thermal surface pasteurization was examined in combination with liquid smoke extracts to reduce and prevent growth of *L. monocytogenes* during shelf life of sensitive RTE meat products. When tested on frankfurters inoculated at  $10^8$  CFU, a liquid smoke extract demonstrated a 0.25-log reduction of *L. monocytogenes*, whereas 1 min in-bag pasteurization (73.9°C/165°F) of inoculated franks gave a 2.9 log reduction. However the combination of liquid smoke and pasteurization gave a 5.25-log reduction that resulted in no detectable *Listeria* by week 3 when tested weekly for 6 weeks of storage at 10°C (50°F). In comparison tests, a 1 sec dip in a clear liquid smoke extract with reduced smoke flavor gave a 5-log reduction on frankfurters when heated a 73.9°C (165°F) for 1 min with no recoverable *Listeria* detected for 10 weeks when stored at 10°C (50°F). When a liquid smoke extract was tested in combination

with prepackage radiant heat pasteurization of deli turkey chubs using a radiant heat oven, grow out of *L. monocytogenes* was retarded but not prevented. A similar study combining liquid smoke treatment with postpackage submerged water pasteurization demonstrated a 2-3 log reduction of *L. monocytogenes* on deli turkey chubs with no grow out during 10 weeks of storage at 6.1°C (43°F). The data demonstrates that one or more liquid smoke extracts combined with a thermal process are capable of providing synergistic reduction of *L. monocytogenes* to achieve a USDA-FSIS Alternative 1 process.

### 3. 2. Introduction

*Listeria monocytogenes* is a Gram-positive, psychotrophic foodborne pathogen that occurs widely in the environment (soil, water, plants), is animal-associated, and consequently finds its way into food processing environments (chillers, drains, floors, equipment, conveyor belts, etc.) (Farber et al., 2000; Anonymous, 2003). Consumption of *L. monocytogenes*-contaminated food may lead to serious infection (i.e., listeriosis). Because of the seriousness of infection, it has been recognized as an important public health problem in the United States. According to the CDC (1999), *L. monocytogenes* had the second highest fatality rate (20%) and hospitalization rate (90%) of all pathogens under surveillance in the USA. Those at greater risk of illness were pregnant women, newborns, and adults with weakened immune systems. Pregnant women were about 20-times more susceptible to listeriosis than typically healthy adults (CDC, 2003).

It is estimated that 2500 illnesses and 500 deaths occur each year in United States due to listeriosis (CDC, 2003). In FDA and FSIS risk assessments of *L. monocytogenes* involvement with RTE meats, deli meat posed the highest per annum risk of illness and death, followed by hotdogs (i.e., frankfurters, wieners, etc) as a moderate public health risk (Anonymous, 2003). The Center for Disease Control and Prevention (CDC) reported that a multistate outbreak between 1998 and 1999, which caused 100 cases and 21 deaths due to contamination of hotdogs and deli meats with *L. monocytogenes* (CDC, 1999). In 2000, 29 cases, 4 deaths, and 3 miscarriages or stillbirths occurred due to consumption of *L.*



*monocytogenes* contaminated deli turkey meat (CDC, 2000). More recently, in northeastern United States, consumption of sliceable turkey deli meat caused 46 cases, 7 deaths, and 3 stillbirths or miscarriages (CDC, 2002).

Several steps have been taken by government regulatory agencies to control *Listeria* and to reduce foodborne contamination by this pathogen. Both USDA-FSIS and FDA have adopted 'zero tolerance' policies on ready-to-eat (RTE) products. USDA-FSIS initiated various incentives (i.e., reduced product testing) if companies were to implement microbial interventions that would reduce risk and increase safety (i.e., Directive 10,240.3). More recently, FSIS published a final rule with 3 'Alternatives' ranging from sanitation alone to control *L. monocytogenes* (Alternative 3), to using postprocess lethality steps or antimicrobial ingredients (Alternative 2), or both (Alternative 1) (Zhu et al, 2005).

In prior studies, we examined several postprocess lethality steps (pre- and post-package pasteurization) that have been accepted by both the RTE processed meat industry and USDA-FSIS as microbial interventions for *L. monocytogenes* in deli meats (Muriana references). In additional studies, we also examined liquid smoke extracts as a surface treatment to provide reduction and control of *L. monocytogenes* during shelf life studies with frankfurters (Gedela et al., 2005). The objective of the present work was to examine the effects of combining the long term benefit of surface treatment with liquid smoke together with the immediate reduction obtained with postprocess pasteurization to prevent product contamination with *Listeria monocytogenes*.

### 3. 3. Materials and Methods

**3. 3. 1. Bacterial strains.** The strains of *L. monocytogenes* used in this study were Scott A-2 (serotype 4b; clinical isolate), V7-2 (serotype ½ a; milk isolate), 39-2 (retail hotdog isolate), and 383-2 (ground beef isolate). Cultures were transferred from frozen stocks stored at -75°C into individual brain heart infusion (BHI) broth tubes at 1% inoculum level, and incubated overnight at 30°C. Overnight cultures were then mixed in equal proportions and 1 ml of mixed culture was used for making dilutions in 0.1% buffered peptone water (BPW) to obtain specific inoculum levels. These strains were resistant to streptomycin (100 µg/ml; Sigma Chemical Co., St. Louis, MO) and rifamycin S/V (10 µg/ml; Sigma) and platings were performed on tryptic soy agar (TSA; Difco, Becton-Dickenson, Franklin Lakes, NJ) containing these antibiotics to exclude the detection of indigenous bacteria from non-sterile food products.

**3. 3. 2. Smoke extracts.** A commercial supplier of liquid smoke condensates (Mastertaste, Inc., Monterey, TN) provided liquid smoke extracts used in this study. The specifications listed for List-A-smoke (Cat. no. 1040071) were 7.0–8.0% titratable acidity, 1.014–1.024 specific gravity, pH 2.0–2.5, and 8.44–8.53 lb/gal density. Zesti-B™ is Zesti-AM2 (Cat no. 1042004) that had been adjusted to pH 4.2–4.4, contained 3.5–5.6% titratable acidity, and had a specific gravity of 1.1–1.2 gm/ml. The AM3 extract (Cat. no. 4000063) used in this study had a pH of 4.1–4.3, 1.095–1.115 specific gravity, and 1.8–2.1% titratable acidity. Liquid

smoke extracts are generally listed as “natural liquid smoke flavor” on ingredient labels. The extract was stored at room temperature and used directly at full strength as received.

**3. 3. 3. Pasteurization trials with several liquid smoke extracts.** Initial postpackage pasteurization trials were performed with retail hotdogs containing lactate/diacetate, but which still allowed growth of *L. monocytogenes* when inoculated at even low levels ( $\sim 10^1$  CFU) (Gedela et al., 2005). To further enhance the likelihood of growth of *L. monocytogenes* on controls, they were boiled for  $\sim 5$  min and then chilled to eliminate indigenous bacteria and/or soluble ingredients at the surface. Hotdogs treated with liquid smoke extract were dipped for 2 min in either List-A-Smoke<sup>™</sup> or Zesti-B<sup>™</sup>. Hotdogs for the various treatments were placed 2 per bag to which a moderately high level of a 4-strain mixture of *L. monocytogenes* was added ( $\sim 1 \times 10^7$  CFU) and then vacuum-sealed. Three treatments were chosen for analysis: 1) smoke alone (plus inoculum), 2) heat alone (plus inoculum), and 3) smoke and heat (plus inoculum). Postpackage pasteurization was done by submersion of packages in hot water at 73.9°C (165°F) for 1 min followed by immersion in an ice slurry. All samples were held at 10°C (50°F) for shelf life testing for 4 (List-A-Smoke) or 6 (Zesti-B) weeks. Postpackage surface pasteurization of hotdogs was performed using a 50-gal (189 liters) steam-injected temperature-controlled circulating water bath (Muriana et al., 2002). All trials were performed in triplicate replications.

**3. 3. 4. Effects of liquid smoke in combination with prepackage pasteurization (radiant heat oven).** The effect of liquid smoke (Zesti-B™) was examined in combination with prepackage pasteurization on the viability of *L. monocytogenes* during shelflife using RTE turkey products (~2 lb. chubs). Samples were given four different treatments, each inoculated with 1 ml (~10<sup>6</sup> CFU/ml) of a 4-strain mixture of *L. monocytogenes* that was pipetted onto product surface, spread with a gloved finger, and vacuum-packaged before shelflife storage. Treatments included: a) controls that were inoculated but not treated with smoke extract or heat, b) samples that were smoke-treated by dipping turkey chubs in liquid smoke for 1 sec and allowed to drip dry for 5 mins before inoculation and packaging, c) samples that were heat treated by passage through a radiant heat, and d) samples that were treated by dipping in smoke extract, inoculated, heated in a radiant heat oven for 60 sec, vacuum-packaged, and stored for shelflife testing. Samples were stored at 6°C (43°F) and plated at day zero and every two weeks thereafter for up to 10 weeks. Packages of turkey chubs were opened, followed by addition of 20 ml of 0.1% BPW, massaged vigorously, and all liquid was withdrawn for purge/rinse volume determination and plating. For prepackage pasteurization a radiant oven (Infrared Grill™, Unitherm Food Systems, Inc., Bristow, OK) was used at a dwell time of 60 sec at high power (480 V, 30 Amp) in our food pathogen pilot plant as described previously (Gande and Muriana, 2003).

**3. 3. 5. Effects of liquid smoke extract in combination with postpackage pasteurization (hot water immersion).** Deli turkey chubs (2-4 lbs) were used for evaluation of liquid smoke (Zesti-B™) in combination with post-package pasteurization for inhibition of *L. monocytogenes*. Post-package surface pasteurization of deli turkey was performed using a 50-gal (189 liters) steam-injected temperature-controlled water bath. Turkey chubs were removed from packaging wrap and given different treatments. Smoke application was done by dipping the products for 1-sec in liquid smoke and allowed to drip dry for 2-5 min and placed in vacuum-packaging bags (heat-only control samples were not dipped in smoke extracts). A mixture of 4-strains of *L. monocytogenes* (~10<sup>6</sup> CFU) was dribbled onto bagged products and then massaged to distribute the inoculum on the samples before vacuum-packaging. A sufficient amount of product samples were treated and then held at 6°C (43°F) for shelflife storage and weekly testing for 10 weeks. All treatments were inoculated samples that included a) no heat or smoke treatment (control #1), b) heating for 60 sec (control #2; without smoke treatment), c) smoke alone (control #3), d) smoke plus 15 sec heating, e) smoke plus 30 sec heating, f) smoke plus 45 sec heating, and g) smoke plus 60 sec heating. All heating was done at 200°F/ 93.33°C by postpackage pasteurization submersion heating (Muriana et al., 2002). When plating samples, products were massaged generously before opening, BPW diluent was added to recover cells, and the volume of package purge was additionally quantitated for adjustment of counts that may have been diluted by the generation of purge during in-bag heating and/or storage.

**3. 3. 6. Comparison of postpackage pasteurization effects of Zesti-B vs. AM-3.** In another series of trials, Zesti-B<sup>™</sup> was compared with a colorless and reduced-flavor extract (AM-3<sup>™</sup>) using hotdogs produced by a local processor without any added lactate/diacetate. These hotdogs were dipped for only 1 sec in the liquid smoke extracts and were given four different treatments. Controls were inoculated, but not treated with either smoke or heat. Pasteurization controls included franks that were inoculated in-bag, vacuum-packaged, and then heated at 73.9°C (165°F) for 1 min. For smoke treatment in combination with pasteurization, hotdogs were dipped in Zesti-B<sup>™</sup> or AM-3<sup>™</sup> for 1 sec, allowed to drip dry, placed in a vacuum bag to which was added 0.5 ml of  $\sim 1 \times 10^5$  CFU/ml inoculum, vacuum sealed, and then pasteurized for 1 min at 73.9°C (165°F). Pasteurized samples were chilled in an ice slurry for 3-5 min before placing at 6°C (43°F) for shelf life storage. Hotdog samples were placed 2 per package and plated at weekly intervals by adding 2-5 ml of 0.1% BPW diluent for resuspending the surface flora. All treatments were done in triplicate replication.

**3. 3. 7. Microbiological analysis.** After the experiment, the products were kept for shelflife study and plated weekly or every two weeks. For sample recovery,, either 2- 5 ml (hotdogs) or 20 ml (turkey chubs) of 0.1% BPW was added to opened sample packages, massaged for a few minutes to resuspend surface bacteria into the rinse buffer, which was then plated. Recovery of the rinse buffer was followed by appropriate serial dilutions and either pour plated or spiral plated, depending on the expected level or organisms. Spiral plating was done

using the Eddy Jet (IUL Instruments, Cincinnati, OH) with pre-poured TSA plates containing the antibiotics streptomycin (100 ug/ml) and rifamycin (10 ug/ml). Plates were then incubated for 48 hrs at 30°C (86°F). Colony counts were obtained using an automatic colony counter (IUL COUNTERMAT FLASH 4.2, IUL Instruments).

**3. 3. 8. Statistical analysis.** All trials were performed in triplicate replications. For most studies, different replications were done on separate days. Different lots of the same product were used when possible. The collected data from three replicates were subjected to one-way repeated measures analysis of variance (RM ANOVA) to determine the differences between the different treatments using Sigma Stat 3.1 (Systat Software, Inc., Richmond, USA). All pair-wise, multiple comparisons were done using the Holm-Sidak method.

### 3. 4. Results and Discussion

Post-process contamination of RTE meats with *L. monocytogenes* has become a major concern because of: (i) the ability of *L. monocytogenes* to grow at refrigerated and abuse temperatures, (ii) a history of outbreaks of listeriosis linked to RTE meat products, (iii) and the ability of these RTE meat products to support the growth of *L. monocytogenes*. For these reasons, antimicrobial chemicals and/or thermal processes have been accepted by USDA-FSIS as post-process technologies that can be used to reduce risk and manufacture safer products (final rule). We recently demonstrated the effectiveness of several liquid smoke extracts in the reduction of *L. monocytogenes* on hotdogs and deli turkey breast (Gedela and Muriana, 2005). In this study, we have examined the combined use of liquid smoke extracts and surface pasteurization as postprocess steps that could possibly achieve alternative 1 status.

**Pasteurization trials with several liquid smoke extracts.** In trials with two liquid smoke extracts, hotdogs treated with heat alone showed recovery of residual levels of *L. monocytogenes* to levels 3-5 logs higher within a few weeks (Fig. 2). When used alone, both smoke extracts demonstrated approximately a 2-log reduction of *Listeria* after 4-6 weeks storage (Zesti-B showed 2-log reduction after only 1 week; Fig. 2). When combined with inpackage pasteurization, the inhibitory effect was exacerbated by heating with both extracts, but especially with Zesti-B<sup>™</sup> whereby a greater reduction was observed for the combined process than from the sum of the individual treatments and no detectable listeria



were observed after 2 weeks , demonstrating a >7-log reduction (Fig. 2B). The hotdogs used in this series of trials were obtained at retail and contained lactate, but were boiled for 5 min and rechilled before use to reduce indigenous flora and remove leachable inhibitory ingredients and held at 10°C (50°F) during storage. Although these procedures were extreme, the smoke extract was still effective in reducing *L. monocytogenes* to undetectable levels. In subsequent trials, we used products that were manufactured without lactate/diacetate and were stored at lower abuse temperatures (6.1°C/43°F).

**Effects of liquid smoke in combination with prepackage pasteurization (radiant heat oven).** In prior work, we demonstrated the utility of pre- and post-package pasteurization of deli meats for reduction of *L. monocytogenes* (Muriana et al., 2002, 2004; Gande and Muriana, 2003). In this study, we have examined the effect of liquid smoke extract in combination with radiant-heat pre-package surface pasteurization of deli turkey manufactured without lactate/diacetate (Fig. 3). *L. monocytogenes* inoculated in untreated controls grew out immediately and in smoke-only treated samples showed a slight (<1-log) reduction before grow out occurred whereas in heat-treated samples, alone or in combination with liquid smoke, more than a 3–log reduction of surface-inoculated *L. monocytogenes* was initially obtained. However, residual *L. monocytogenes* in the heat-treated samples grew out rapidly and increased by ~3 logs between weeks 1 and 2 while samples from the combined (smoke+heat) treatment were delayed nearly 4 weeks to reach the same level of grow out (Fig. 3). A possible explanation for the

growth of this pathogen could be that pre-package pasteurization using radiant heat directly heats the products' exposed surface resulting in a 'burning off' of some of the active volatile agent (possible volatilization of the volatile attributes of liquid smoke exposed to heating) such that recovery of cells not killed by heat may occur. In prior studies with pre-package pasteurization alone, we identified conditions that could completely eliminate up to 100 CFU of *L. monocytogenes* inoculated onto product surface, but at higher inoculation levels some cells may survive the heat treatment (Gande and Muriana, 2003). It is also possible that deli turkey may not absorb as much of the liquid smoke product in our 1-sec dip treatment that was found to be effective for frankfurters (Fig. 2; Gedela et al., 2005) and perhaps length of dip treatments should be examined for each different class of RTE product.

**Effects of liquid smoke extract in combination with postpackage pasteurization (hot water immersion).** We also examined liquid smoke treatment in combination with post-package pasteurization of deli turkey by hot water immersion as this process does not subject exposed product surfaces, but rather is an in-bag heat treatment and is more consistent with how we heat-treated frankfurters in our earlier trials (Fig. 2). Again, untreated inoculated controls grew immediately while liquid smoke treatment alone resulted in a 1-log reduction before grow out occurred (after 2 weeks) and heat treatment alone for the maximum heating time used in the combination trials resulted in ~1.3-log reduction that also grew out immediately (Fig. 4). However, in contrast to our

results with radiant heat pasteurization, almost all of our combination treatments (liquid smoke + 30-, 45-, and 60-sec postpackage pasteurization) resulted in >2-log reduction of *L. monocytogenes* that was maintained for 10 weeks without grow out except for the 15-sec heat treatment which grew out after 8 weeks (Fig. 4). The results are an improvement over those obtained with liquid smoke/radiant heat combination treatments as well as those obtained with postpackage pasteurization alone which often requires as much as 2-4 min of submersion heat treatment by companies that adopt this process to achieve appreciable reduction levels for *L. monocytogenes*. Unfortunately, the generation of purge is a byproduct of prolonged in-bag heat treatment and the data suggests that reduced heat treatments may be accommodated by combination processes with liquid smoke or other similar-acting antimicrobials (Fig. 4). The heat treatments found effective in Fig. 4 are not much more than what can be obtained with an extended shrink process. The data presented here is of greater significance when considering practical levels (i.e., lower) of *L. monocytogenes* that might be acquired if in-house contamination were to occur. However, the use of low inoculum levels has disadvantages of not showing the trend of inhibition that can only be observed using higher inoculation levels.

### **Comparison of postpackage pasteurization effects of Zesti-B vs. AM-3.**

Because of the potential use of antimicrobial liquid smoke extracts on a greater range of products than just those in which smoke flavor is preferred or a darkened color may not be desirable, we compared the inhibition of the liquid

smoke extract used above (Zesti-B™) with a liquid smoke extract that was colorless and had reduced smoke flavor (Am-3™). Hotdogs used in this study were manufactured without lactate or diacetate and were dipped in either liquid smoke extract for 1 sec before being vacuum packaged with added inoculum of *L. monocytogenes* and subjected to 60-sec inbag pasteurization at 73.9°C (165°F). Untreated inoculated controls grew out immediately during storage while heated franks showed a 1.7-log reduction of *L. monocytogenes* before emergence and grow out within 1 week (Fig. 5). However, both liquid smoke extracts showed similar *listeria* reductions observed earlier (Fig. 2B) whereby initial levels were reduced to undetectable levels that remained below levels of detection for the entire 10 weeks of storage. The difference between the initial dramatic reduction observed here (Fig. 5) and the slower decline observed in Fig. 2B may be explained by the lower inoculation level used (i.e., 10<sup>5</sup> vs. 10<sup>7</sup> CFU/ml) and recovered from samples. It is not clear why we obtain such drastic reductions with frankfurters as opposed to deli turkey although product type and/or size could be suspect causes of the different reactions to the combination processes used in our study (i.e., frankfurters vs. 2-4 lb turkey chubs whereby the heat process does not penetrate as deep with such an underlying cold sink on the larger turkey chubs?).

Our data demonstrates the inhibitory effect of liquid smoke on *L. monocytogenes* was enhanced by pre- and post-package pasteurization of RTE meat products. Although either process can certainly have utility on their own, the data presented herein demonstrates that the combination of heat and liquid

smoke extracts may provide for reduced heating regimens that are usually observed with individual surface pasteurization processes. The ability to reduce and suppress outgrowth of *L. monocytogenes* suggests that such processes may be considered as Alternative 1 processes for USDA-FSIS RTE processed meats.

### **3. 5. Acknowledgements**

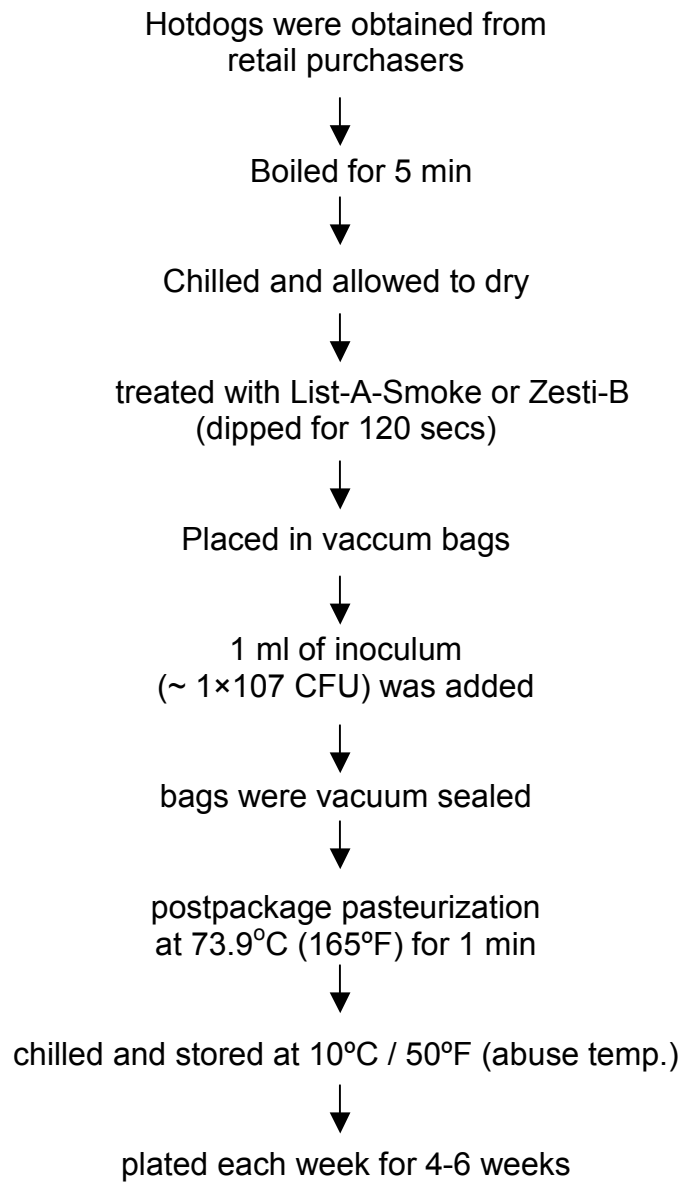
This research was partially funded by the Oklahoma Agricultural Experiment Station, Oklahoma State University, Stillwater, OK, and the American Meat Institute Research Foundation. I would like to thank Mastertaste Inc., Bar-S Foods Inc., and the Jennie-O Turkey Store for providing product, technical, and/or financial assistance during the course of this project. I also thank Sunita Macwana, Rachel Wright, and William Robertson for contributions in conducting the IR Grill and post-package pasteurization combination trials as designed by me and my advisor.

### 3. 5. References

- Anonymous, 2003. FSIS risk assessment for *Listeria monocytogenes* in deli meats. Draft FSIS *Listeria* risk assessment.  
<http://www.fsis.usda.gov/OPPDE/rdad/FRPubs/97-013F/Pg1-31.pdf>
- Center for Disease Control and prevention (CDC). 2003. Listeriosis, Technical information.  
[http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis\\_t.htm](http://www.cdc.gov/ncidod/dbmd/diseaseinfo/listeriosis_t.htm)
- Center for Disease Control and prevention (CDC). 2002. Multistate outbreak of listeriosis---Northeastern United States. *Morb. Mortal. Wkly Rep.* 51: 950-951.
- Centers for Disease Control and Prevention (CDC). 2000. Multistate outbreak of listeriosis --- United States, 2000. *Morb. Mortal. Wkly Rep.* 49: 1129-1130.
- Centers for Disease Control and Prevention (CDC). 1999. Multistate outbreak of listeriosis --- United States, 1998-1999. *Morb. Mortal. Wkly Rep.* 51: 1117-1118
- Centers for Disease Control and Prevention (CDC). 1998. Multistate outbreak of listeriosis --- United States, 1998. *Morb. Mortal. Wkly Rep.* 50: 1085-1086.
- Farber, M. J., E. M. Daley, M.T. Mackie and B. Limerick. 2000. A small outbreak of listeriosis potentially linked to the consumption of imitation crabmeat. *Lett. Appl. Microbiol.* 31: 100-104
- Gande, N., and P. M. Muriana. 2003. Prepackage surface pasteurization of ready-to-eat meats with a radiant heat oven for reduction of *Listeria monocytogenes*. *J. Food Prot.* 66: 1623-1630.
- Gonulalan, Z., A. Kose, and H. Yetim. 2003. Effect of liquid smoke on quality characteristics of Turkish standard smoked beef tongue. *Meat Sci.* 66: 165-170.
- Messina, M. C., A. H. Ahmad, A. J. Marchelo, P. C. Gerba, and W. M. Paquette. 1988. Effect of liquid smoke on *Listeria monocytogenes*. *J. Food Prot.* 51: 629-631.
- Muriana, P.M., W. Quimby, C. A. Davidson, and J. Grooms. 2002. Postpackage pasteurization of ready-to-eat deli meats by submersion heating for reduction of *Listeria monocytogenes*. *J. Food Prot.* 65: 963-969.
- Seeliger, H. P. R., and D. Jones. 1993. *Listeria*. P: 1235-1245. In. J. G. Holt (Ed), *Bergey's Manual of Systematic Bacteriology*. 9<sup>th</sup> ed. The William & Wilkins. Baltimore.

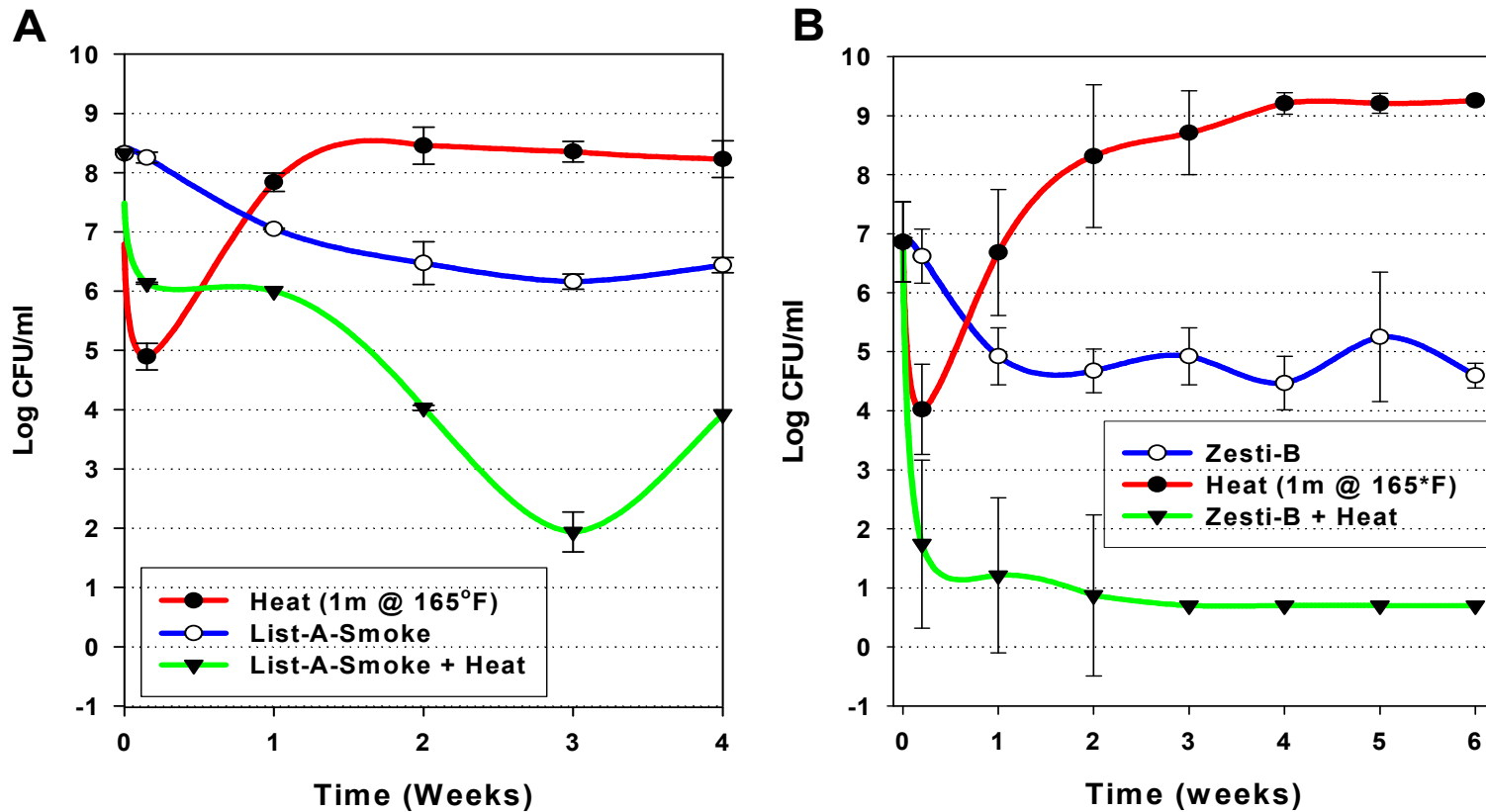
Thurette, J., J. M. Membre, L. H. Ching, R. Tailliez, and M. Catteau. 1998. Behavior of *Listeria spp.* in smoked fish products affected by liquid smoke, NaCl concentration, and temperature. *J. Food Prot.* 61: 1475-1479.

Zhu, M., M. Du, J. Cordray, and D. V. Ahn. 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Comprehensive review in Food Sci. and Technol.* 4: 34-42.

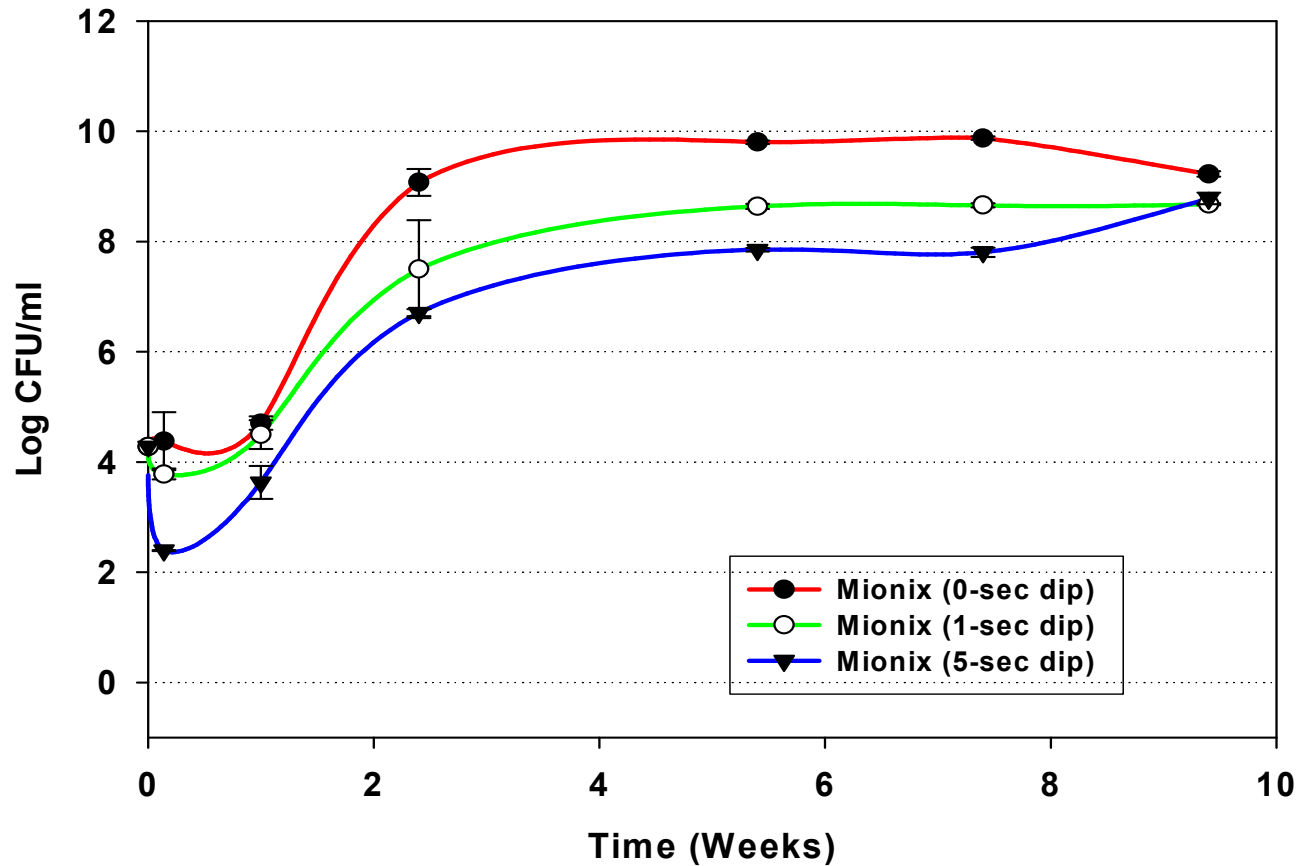


**Figure 1.** Diagrammatic representation of evaluation of 2 liquid smoke extracts for anti-*Listerial* activity on hotdogs

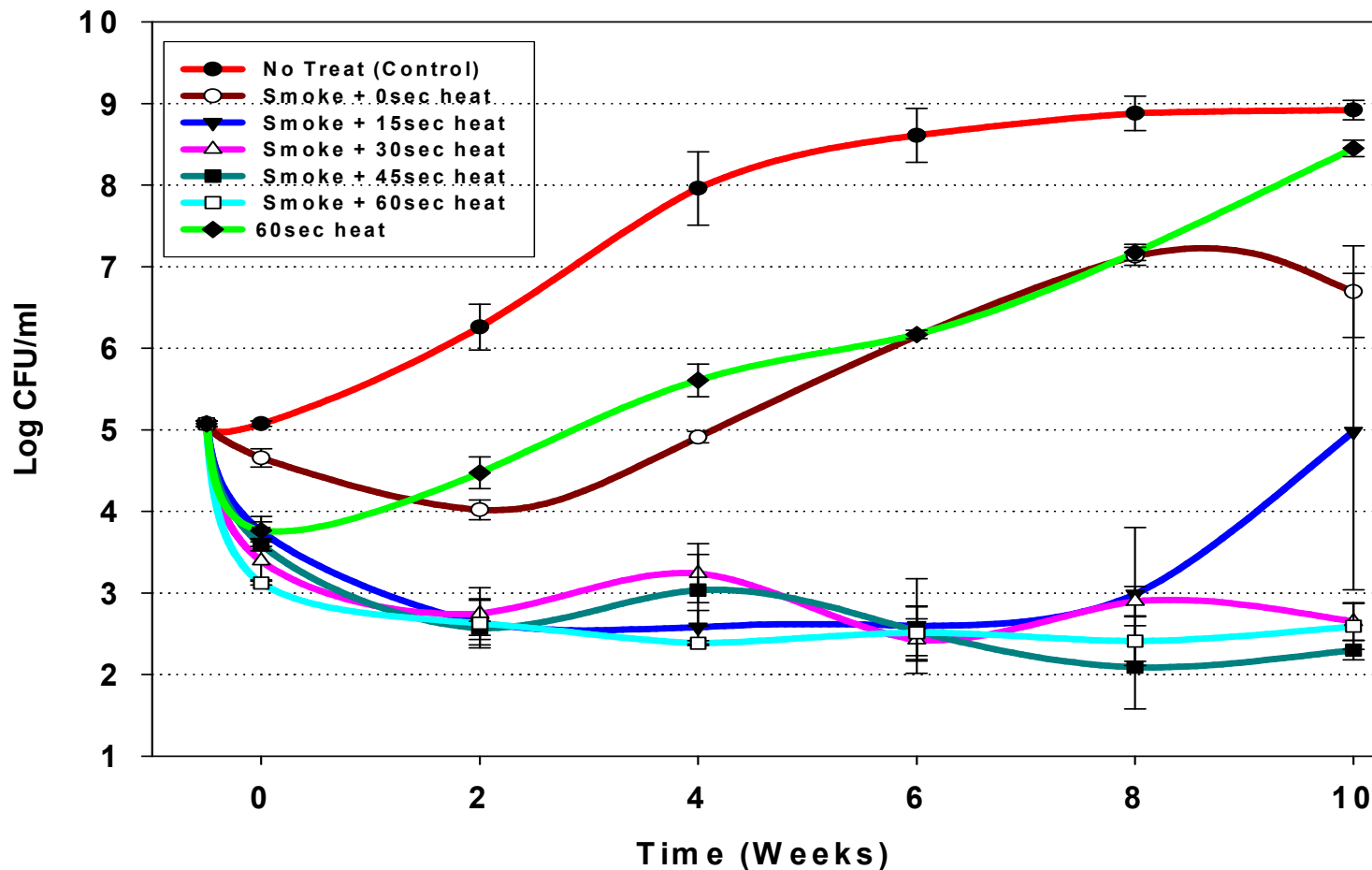




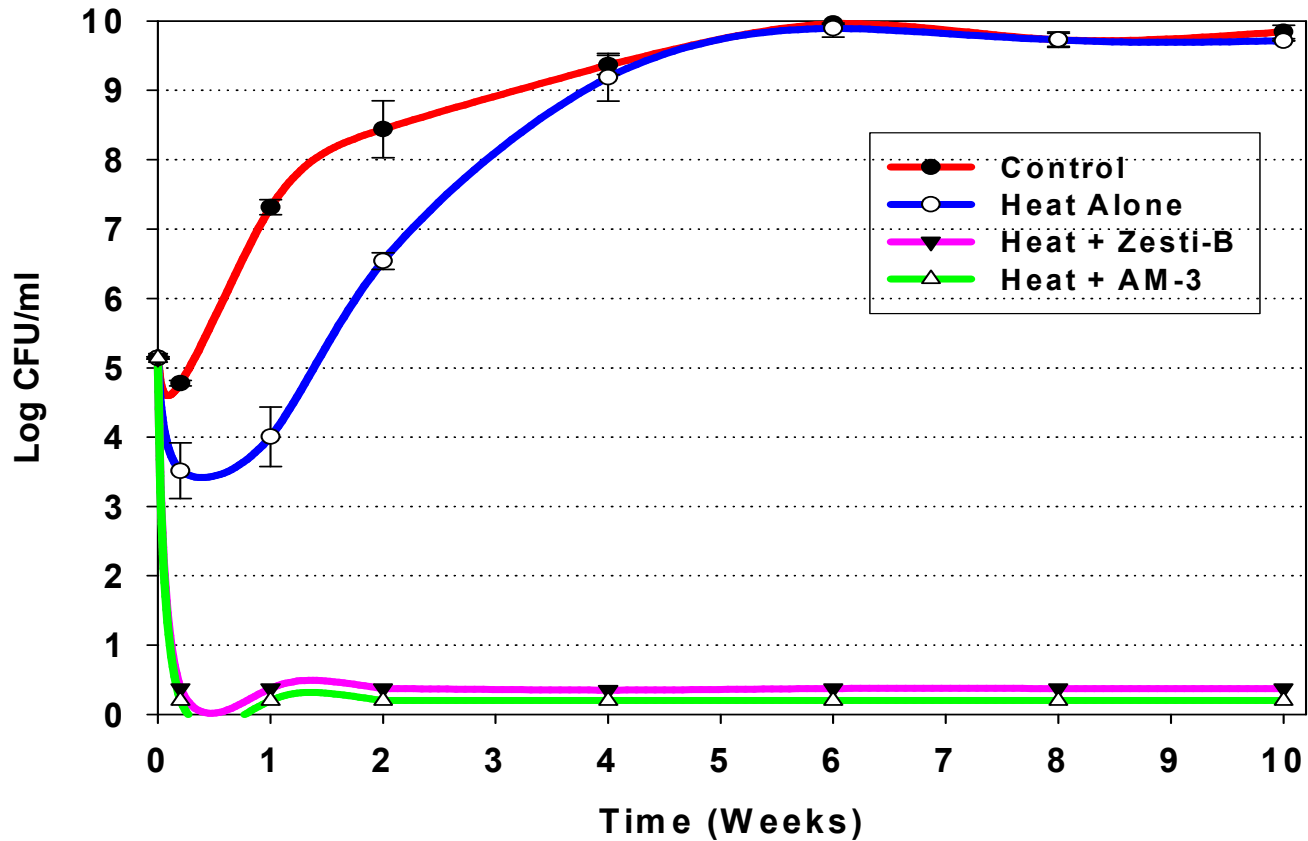
**Figure 2.** Evaluation of 2 liquid smoke extracts for anti-*Listerial* activity on hotdogs. Panel A, hotdogs with either List-a-Smoke™ alone, heat treatment alone (1 min @ 165°F), or List-a-Smoke™ in combination with heat treatment (1 min @ 165°F). Hotdogs were dipped for 2 min in List-a-Smoke™ and dripped dry for 5 min before use. Hotdogs were inoculated by adding 1 ml of a 4-strain mixture of *L. monocytogenes* into packaging bags before vacuum-packaging and held at 50°F (abuse temperature) for shelf-life testing. Panel B, same as Panel A except using Zesti-B™.



**Figure 3.** Liquid smoke (Zesti-B™) in combination with prepackage pasteurization of 2-4 lb deli turkey breasts manufactured without lactate/diacetate. Deli turkey chubs were immersed for 1-sec in liquid smoke extract, allowed to drip-dry for 10 sec, and 1-ml of a 4-strain mixture of *L. monocytogenes* was dribbled over the surface and spread with a gloved-finger. The products were then heated for 60- sec in a radiant heat oven (~500°F air temperature) used for prepackage pasteurization of RTE deli meats. All treatments were performed in triplicate replications and error bars represent standard deviation from the mean.



**Figure 4.** Liquid smoke (Zesti-B™) in combination with post-package pasteurization of deli turkey breast. Deli turkey chubs were immersed for 1-sec in liquid smoke extract and allowed to drip-dry for 10 sec. Samples were then bagged, a 4-strain mixture of *L. monocytogenes* was added, and products were pasteurized at 200°F for -, 15-, 30-, 45-, and 60-sec, including a 60-sec heat-treatment without smoke extract. All treatments were performed in triplicate replications.



**Figure. 5** Effect of Zesti-B™ (brown) vs. AM-3™ (clear) liquid smoke in combination with heat against *Listeria monocytogenes*. Hotdogs treated either with Zesti-B™ along with heat treatment (1 min @ 165°F), or with AM-3 in combination with heat treatment (1 min @ 165°F). Hotdogs were dipped for 1 sec in liquid smoke (Zesti-B™ or AM-3™), inoculated by adding 0.5-ml of a 4-strain mixture of *L. monocytogenes* into packaging bags before vacuum packaging and held at 43°F (abuse temperature) for shelf-life testing.

**APPENDIX**

**EFFECT OF MIONIX AGAINST *LISTERIA MONOCYTOGENES*  
ON READY-TO-EAT MEATS**

### **Treatment of hotdogs with Mionix RTE-01:**

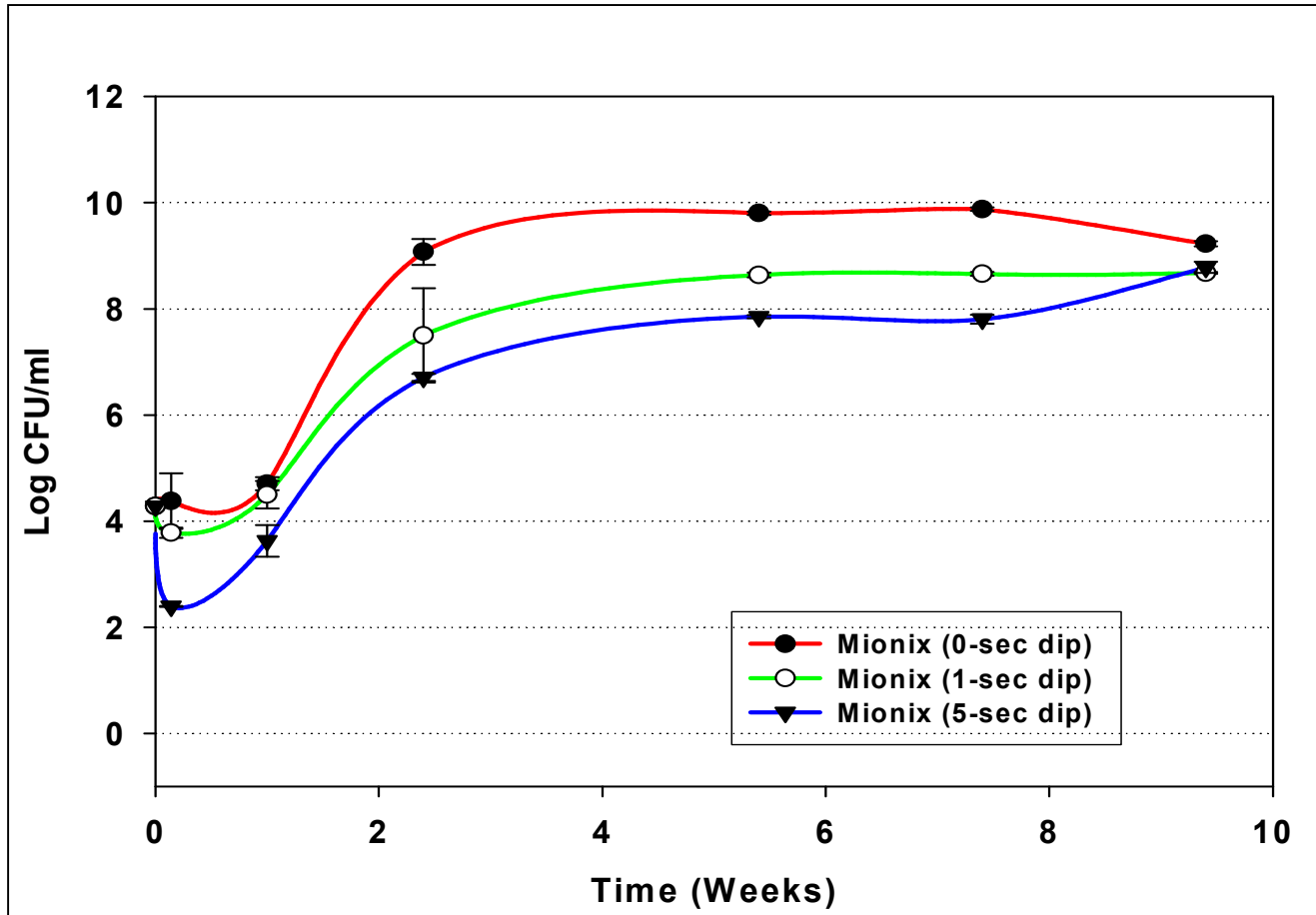
Mionix (Safe2O<sup>®</sup> RTE-01) used in this study is an antimicrobial composed of a mixture of water, phosphate, lactic acid, and acid calcium sulfate and has a boiling point of 104°C, specific gravity of 1.06-1.1, and pH of <2.0. Mionix RTE-01 has a post-lethality effect and continues to inhibit microbial growth and extend shelflife well after treatment.

**Dipping of hotdogs for 30 sec in Mionix RTE-01.** Hotdogs used in this study were manufactured without lactate and diacetate. Hotdog samples were pre-inoculated with a 4-strain mixture of *L. monocytogenes* at a level of 10<sup>5</sup> CFU/ml and dipped in either buffer or RTE-01 for 30 sec and allowed to drip dry for 5-10 sec, samples were thoroughly massaged before vacuum packaging, and then stored at 6°C (43°F) for 10 weeks. Initial samples were tested before (control) and after dip treatments (control and experimental) and subsequent samples were retrieved weekly or biweekly for shelflife testing. Data points fig. 1 represent the mean of triplicate replications and error bars represent standard deviation from mean. There is a significant difference ( $P < 0.001$ ) between treatments and the controls. The results showed that dipping of pre-inoculated samples into RTE-01 for 30 sec was very effective.

**Examination of shorten dip times.** Since the 30 Sec dipping in the Mionix RTE-01 was very effective in reducing the growth of *L. monocytogenes*, we have started to examine Mionix RTE-01 at shorter dip times than the 30 sec (Fig. 2). In

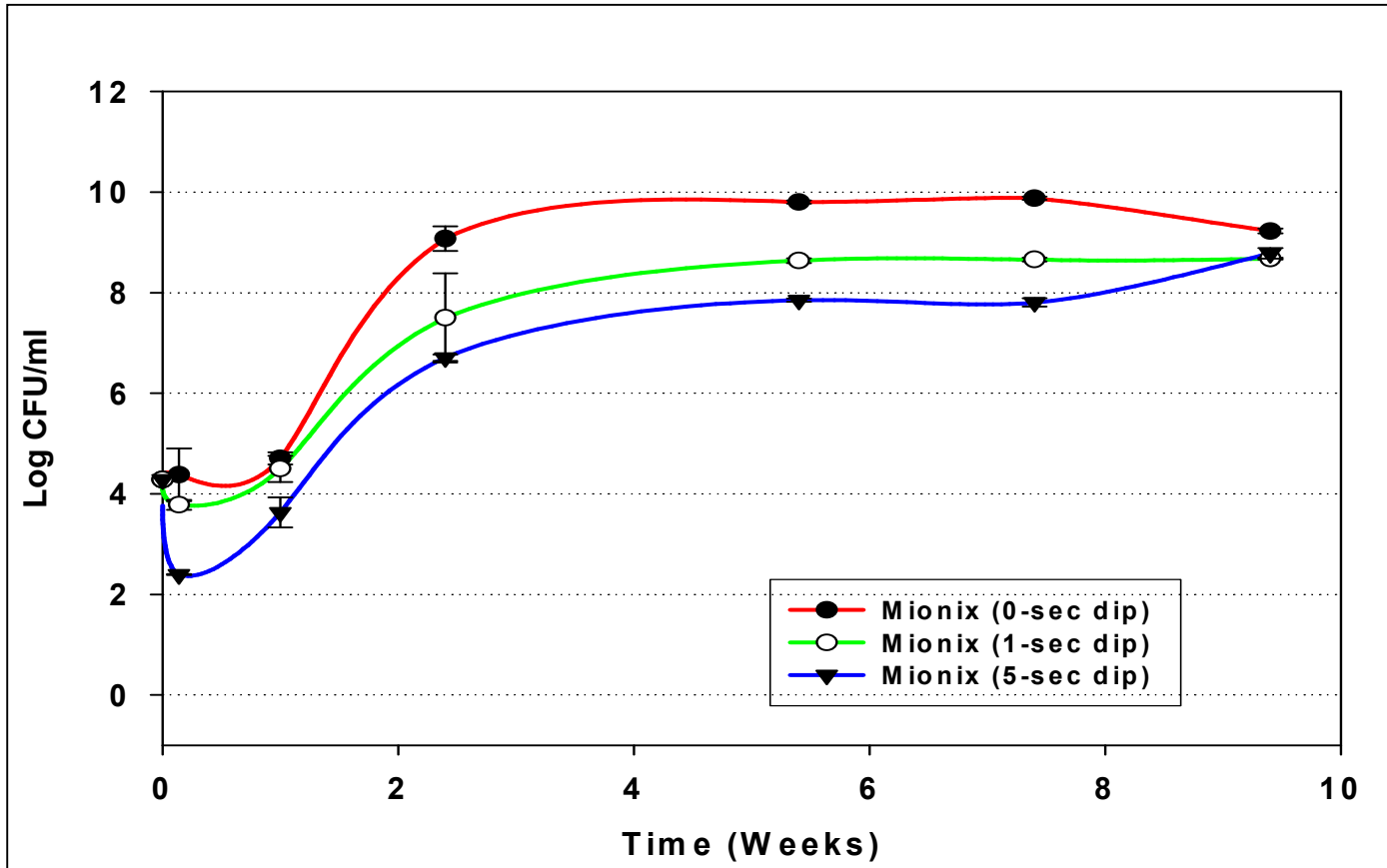
this study, both pre-inoculation (dipped in the inoculum first, and then in RTE-01) and post-inoculation (dipped in RTE-01 first and then adding inoculum) was performed. When pre-inoculated hotdogs were treated with a high-level inoculum ( $\sim 10^6$  CFU *L. monocytogenes*) and then dipped in RTE-01 for 0-, 1-, or 5-sec, we obtained reductions with all 3 treatments commensurate with the level of treatment, and then observed recovery during subsequent shelflife periods. Products were sampled at 1 hr, 24 hr, and 1 week and then bi-weekly. The results indicate that although the 5-sec dip in RTE-01 gave a  $>3.5$ -log reduction from the inoculum, recovery occurred within a short time to reach very high levels.

The above experiment was repeated with three different inoculation levels,  $10^2$ ,  $10^3$ , and  $10^4$  CFU/ml (Fig. 3) in which hotdogs (without lactate and diacetate) were pre-dipped in RTE-01 and then inoculum was added to the bags before packaging and followed by shelflife testing at 43°F. By the end of the 10<sup>th</sup> week, the only treatment that was able to prevent the outgrowth of *L. monocytogenes* was a 5-sec treatment which was only effective against the lowest level used.

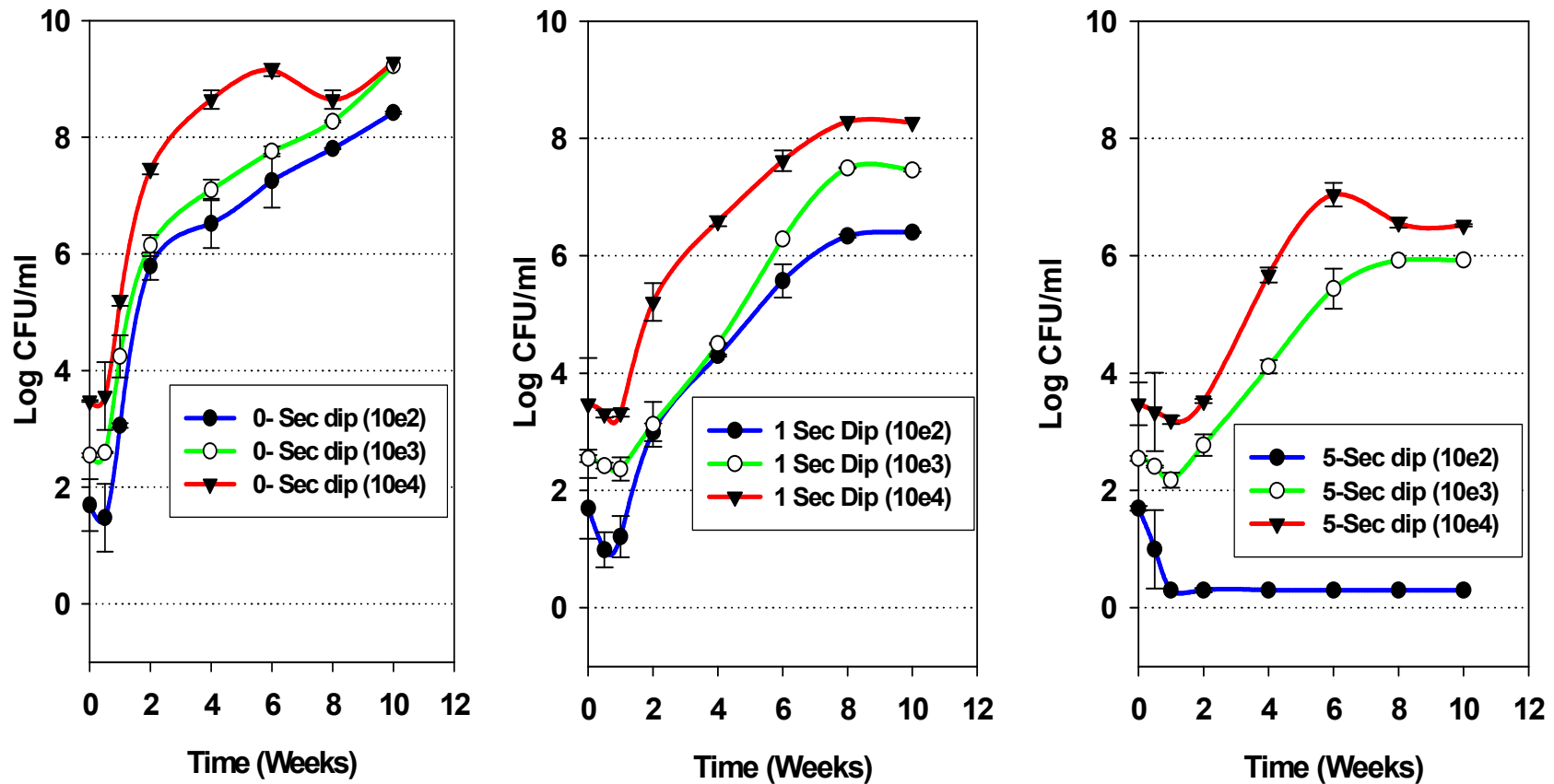


**Figure 1.** Hotdog samples (without lactate and diacetate) were pre-inoculated with a 4-strain mixture of *L. monocytogenes* ( $10^5$  CFU/ml) and dipped in either buffer or RTE-01 for 30 sec and allowed to drip dry for 5-10 sec, vacuum packaged, and stored at 6°C (43°F) for 10 weeks. Data points fig. 1 represent the mean of triplicate replications and error bars represent standard deviation of the mean.





**Figure 2.** Pre-inoculated hotdogs were treated with high-level inoculum ( $\sim 10^6$  CFU *L. monocytogenes*) and then dipped in RTE-01 for 0-, 1-, or 5-sec. Products were sampled at 1 hr, 24 hr, and 1 week and then bi-weekly.



**Figure 3.** Hotdogs were pre dipped in RTE-01 for 0, 1, and 5 sec and inoculated with three different inoculation levels,  $10^2$ ,  $10^3$ , and  $10^4$  CFU/ml. Then vacuum packaged and kept for shelflife testing at 43°F.

VITA

SARITHA GEDELA

Candidate for the Degree of

Master of Science or Arts

Thesis: APPLICATION OF LIQUID SMOKE ALONE AND IN COMBINATION  
WITH PRE-AND POST-PACKAGE PASTEURIZATION AGAINST *LISTERIA*  
*MONOCYTOGENES* ON READY-TO-EAT MEATS

Major Field: Food Science

Biographical:

Personal Data

Born in India, AP, on March 23, 1978, the daughter of Yerram  
Naidu and Indira Devi.

Education

Completed the graduation requirements for the degree of  
Bachelor of Science in Home Science in October 2000.  
Completed the requirements for the Masters of Science degree  
with a major in Food Science at Oklahoma State University in  
December 2005.

Experience

Employed by Oklahoma State University under Dr. Peter M.  
Muriana as a graduate assistant; Oklahoma State University,  
Department of Animal Science, 2003 to present.

Name: Saritha Gedela

Date of Degree: December, 2005

Institution: Oklahoma State University

Location: Stillwater, Oklahoma

Title of Study: APPLICATION OF LIQUID SMOKE ALONE AND IN  
COMBINATION WITH PRE-AND POST-PACKAGE PASTEURIZATION  
AGAINST *LISTERIA MONOCYTOGENES* ON READY-TO-EAT MEATS

Pages in Study: 99

Candidate for the Degree of Master of Science

Major Field: Food Science

*L. monocytogenes* has been troublesome for many RTE meats. Our objectives were to examine the effect of liquid smoke extracts on the inhibition of *L. monocytogenes* on hotdogs (used as a meat matrix) when used alone or in combination with postpackage pasteurization. Liquid smoke extracts were able to reduce and control growth of *L. monocytogenes* on franks when treated for as long as 90 sec and as short as 1 sec or when dropped through an atomized mist produced by a pressurized spray canister. Surface application of liquid smoke by dipping or spraying may inhibit the growth of *L. monocytogenes* on hotdogs during shelf life. Thermal surface pasteurization was also examined in combination with liquid smoke extracts against *L. monocytogenes* and demonstrated that one or more liquid smoke extracts combined with a thermal process are capable of providing synergistic reduction of *L. monocytogenes* to achieve a USDA-FSIS Alternative 1 process.

ADVISER'S APPROVAL: Dr. Peter M Muriana

---