

5-2012

# Utilization of Natural Green Tea and Grape Seed Extracts and Nisin to Reduce Conventional Chemical Preservatives and to Inhibit the the Growth of *Listeria Monocytogenes* in Ready to Eat Low and High Fat Chicken and Turkey Hotdogs

Amara Venkata Sunil Perumalla  
*University of Arkansas*

Follow this and additional works at: <http://scholarworks.uark.edu/etd>

 Part of the [Food Chemistry Commons](#), [Food Processing Commons](#), and the [Pathogenic Microbiology Commons](#)

---

## Recommended Citation

Perumalla, Amara Venkata Sunil, "Utilization of Natural Green Tea and Grape Seed Extracts and Nisin to Reduce Conventional Chemical Preservatives and to Inhibit the the Growth of *Listeria Monocytogenes* in Ready to Eat Low and High Fat Chicken and Turkey Hotdogs" (2012). *Theses and Dissertations*. Paper 303.

**UTILIZATION OF NATURAL GREEN TEA AND GRAPE SEED EXTRACTS AND NISIN TO REDUCE CONVENTIONAL CHEMICAL ANTIMICROBIALS AND TO INHIBIT THE GROWTH OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT LOW AND HIGH FAT CHICKEN AND TURKEY HOTDOGS**

**UTILIZATION OF NATURAL GREEN TEA AND GRAPE SEED EXTRACTS AND  
NISIN TO REDUCE CONVENTIONAL CHEMICAL ANTIMICROBIALS AND TO  
INHIBIT THE GROWTH OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT  
LOW AND HIGH FAT CHICKEN AND TURKEY HOTDOGS**

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy in Food Science

By

AmaraVenkata Sunil Perumalla  
Acharya N.G.R. Agricultural University  
Bachelor of Veterinary Science and Animal Husbandry, 2004  
University of Arkansas  
Master of Science in Poultry Science, 2007

May 2012  
University of Arkansas

## ABSTRACT

Ready-to-eat meat (RTE) products such as hotdogs represent a popular segment in convenience food purchases. Increased demand has led the processors to extend the shelf life by minimizing lipid oxidation and post-processing contaminants such as *Listeria monocytogenes*. There is a growing interest in the food processors and consumers regarding the use of ‘natural alternatives’ in place of synthetic food additives to control the growth of foodborne pathogens and (or) lipid oxidation. In recent years, green tea (GTE) and grape seed extracts (GSE) are increasing choices as they have demonstrated antioxidant as well as antimicrobial properties in various food applications. Therefore, the main objective of this research study is to reduce conventional chemical preservatives [potassium lactate (PL) and sodium diacetate (SD)] by natural plant extracts (GTE and GSE) along with nisin and EDTA combinations.

Optimal levels of GTE (0.3 %) and GSE (0.22 %) partially replaced PL (1.5 %) and SD (0.11 %) and demonstrated more growth inhibition (2.0 log cfu/g) of *L. monocytogenes* compared to commercial hotdog formulations (2.0 % PL and 0.11 % SD; 0.9 log cfu/g). Post-process heat treatment intervention inactivated *L. monocytogenes* in all the samples regardless of the chemicals, plant extracts alone and their combination at day zero. However, in chicken hotdogs, survivors were observed by day 12 and grew over time until spoilage (28 days). In hotdog samples without heat treatment, maximum growth inhibitions (approximately 2.0 log cfu/g) were observed in the treatments having combination of chemical preservatives and plant extracts. Addition of GTE and GSE inhibited the lipid oxidation (no detectable levels of hexanal) until the 6<sup>th</sup> week of storage (4 °C). Consumer panelists observed no significant differences ( $P > 0.05$ ) in all sensory attributes except texture ( $P < 0.05$ ; higher scores) in the hotdogs formulated with chemical preservatives and plant extracts that would improve consumer acceptability. Results

from the study shows that GTE (0.35 %) and GSE (0.22 %) are potential natural alternatives that can partially replace conventionally used chemical preservatives without affecting physicochemical and sensory attributes of meat products while having positive impact on consumers by providing additional health benefits.

This dissertation is approved for recommendation  
to the Graduate Council.

Dissertation Director:

---

Dr. Navam S. Hettiarachchy

Dissertation Committee:

---

Dr. Steven C. Ricke

---

Dr. John Marcy

---

Dr. Ruben Morawicki

---

Dr. Edward E. Gbur

---

Dr. Michael F. Slavik

## DISSERTATION DUPLICATION RELEASE

I hereby authorize the University of Arkansas Libraries to duplicate this dissertation when needed for research and/or scholarship.

Agreed \_\_\_\_\_  
**AmaraVenkata Sunil Perumalla**

Refused \_\_\_\_\_  
**AmaraVenkata Sunil Perumalla**

## **ACKNOWLEDGMENTS**

I would like to thank my major professor, Dr. Navam Hettiarachchy for her motivation, mentorship, and funding to pursue my doctoral program. I also would like to extend my gratitude to my committee members for their valuable input and guidance as needed. Thanks to all my fellow lab colleagues for their help and making my graduate program an enjoyable and good learning experience. Finally, I appreciate and so grateful to my wife for her patience during this journey.

**STAY FOOLISH !! STAY HUNGRY!!**

## **DEDICATION**

This dissertation is dedicated to my parents Sri Lakshmi Perumalla and Kanaka Mallikharjuna Rao Perumalla and my wife and son (Sireesha Boganatham and Sameep Perumalla) for all their support and motivation to pursue my doctoral program

## TABLE OF CONTENTS

### I. CHAPTER I

INTRODUCTION .....	1
OBJECTIVES .....	7
REFERENCES .....	9

### II. LITERATURE REVIEW

<i>Listeria monocytogenes</i> .....	12
Hotdogs/Frankfurters .....	15
Chemical Composition of Chicken and Turkey meat .....	16
Control Measures to Reduce Foodborne Pathogens .....	19
Lactates and Diacetates .....	20
Plant Extracts as Antimicrobial agents .....	22
Nisin .....	29
Post Processing Intervention Strategies .....	32
Multiple Hurdle Technology Approach .....	34
Lipid Oxidation .....	42
Physicochemical Properties .....	46
Sensory Properties .....	47
Potential Applications of Green Tea and Grape Seed Extracts In Food Products...	52
References .....	58

### III. EFFECT OF POTASSIUM LACTATE AND SODIUM DIACETATE COMBINATION TO INHIBIT *LISTERIA MONOCYTOGENES* IN LOW AND HIGH FAT CHICKEN AND TURKEY HOTDOG MODEL SYSTEMS

Abstract .....	83
Introduction .....	84

Materials and Methods .....	86
Results and Discussion .....	90
Conclusions .....	95
References.....	96
<b>IV EFFECT OF REDUCING POTASSIUM LACTATE, SODIUM DIACETATE AND INCORPORATING PLANT EXTRACTS, NISIN AND EDTA COMBINATIONS ON INHIBITING <i>LISTERIA MONOCYTOGENES</i> INOCULATED IN HOTDOG MODEL SYSTEM</b>	
Abstract .....	105
Introduction .....	106
Materials and Methods .....	108
Results and Discussion .....	111
Conclusions .....	114
References.....	116
<b>V EFFECT OF PARTIAL REPLACEMENT OF POTASSIUM LACTATE AND SODIUM DIACETATE BY NATURAL GREEN TEA AND GRAPE SEED EXTRACTS AND POST-PACKAGING THERMAL TREATMENT ON THE GROWTH OF <i>LISTERIA MONOCYTOGENES</i> IN HOTDOG MODEL SYSTEM</b>	
Abstract .....	122
Introduction .....	123
Materials and Methods .....	125
Results and Discussion .....	130
Conclusions .....	135
References.....	136
<b>VI EFFECT OF PARTIAL REPLACEMENT OF POTASSIUM LACTATE AND SODIUM DIACETATE BY NATURAL GREEN TEA AND GRAPE SEED EXTRACTS ON PHYSICOCHEMICAL AND SENSORY PROPERTIES OF HOTDOGS</b>	

Abstract .....	145
Introduction .....	146
Materials and Methods .....	148
Results and Discussion .....	153
Conclusions.....	159
References.....	160
Appendix I .....	170

## LIST OF PUBLICATIONS

1. Perumalla, A.V.S and Navam. S. Hettiarachchy. 2011. Green tea and grape seed extracts – Potential applications in food safety and quality. *Food Res Int.*, 44 (4):827-839.

## LIST OF TABLES

- Table 2.1 Chemical composition of Chicken and Turkey meat
- Table 2.2 Summary of research findings involving green tea and grape seed extracts as antimicrobial components in multiple hurdle approach in various model systems.
- Table 3.1A Percent moisture, protein, fat and residual nitrite determined in low and high fat chicken and turkey hotdogs (No lactate and diacetate).
- Table 3.1B pH values determined in low and high fat chicken and turkey hotdog samples
- Table 4.1 Treatments showing various combination levels of potassium lactate (PL) and sodium diacetate (SD) alone and in combination with green tea extract (GTE), grape seed extract (GSE), nisin and EDTA used in this study.
- Table 5.1 Water activity and pH determined for low and high fat chicken and turkey hotdog samples.

## LIST OF FIGURES

- Figure 2.1 Structure of major tea catechins present in green tea extract
- Figure 2.2 Chemical structures of some phenolic compounds from grape seeds.
- Figure 2.3 Structure and chemical structures of nisin.
- Figure 2.4 Transmission electron microscopy (TEM) of *L. monocytogenes* treated by nisin combined with either phenolics or grape seed extract (GSE) in TSBYE medium at 37 °C.
- Figure 3.2. Effect of Potassium Lactate (PL) and Sodium Diacetate (SD) combination against *Listeria monocytogenes* in (A) low fat and (B) high fat chicken hotdogs vacuum packed and stored at 4 °C.
- Figure 3.3 Effect of Potassium Lactate (PL) and Sodium Diacetate (SD) combination against *Listeria monocytogenes* in (A) low fat and (B) high fat turkey hotdogs vacuum packed and stored at 4 °C.
- Figure 4.1 Effect of reducing potassium Lactate (PL) and Sodium Diacetate (SDA) by plant extracts, nisin and EDTA against *Listeria monocytogenes* in (A) low and (B) high fat chicken hotdogs.
- Figure 4.2 Effect of reducing potassium Lactate (PL) and Sodium Diacetate (SDA) by plant extracts, nisin and EDTA against *Listeria monocytogenes* in (A) low and (B) high fat turkey hotdogs.
- Figure 5.1 Post-packaging thermal treatment of vacuum packed bags of hotdog samples used in the study. Vacuum-pack bags attached to the wire-rack to support and for immersion in the hot water bath (A, B, C). Hotdog samples subjected to heat treatment in water bath shown at different views (D, E, F).
- Figure 5.2 Effect of partial replacement of potassium lactate and sodium diacetate by natural green tea and grape seed extracts and thermal inactivation to inhibit the growth of *Listeria monocytogenes* in low (A) and high fat (B) chicken hotdogs.
- Figure 5.2 Effect of partial replacement of potassium lactate and sodium diacetate by natural green tea and grape seed extracts and thermal inactivation to inhibit the growth of *Listeria monocytogenes* in low (A) and high fat (B) turkey hotdogs.

# CHAPTER I

## INTRODUCTION

The Centers for Disease Control and Prevention (CDC) estimate that in the U.S., each year foodborne illness causes forty-eight million cases of foodborne diseases with 128,000 hospitalizations and 3,000 deaths (CDC, 2011). Foodborne illness caused by the consumption of contaminated foods has worldwide economic and public health impacts. Foodborne pathogens constitute an ominous threat to the consumer and food industry alike, as influenced by demographics, industrialization and centralization of food production supply, travel and trade, microbial evolution and adaptation (Tauxe, 1997; WHO, 1997). The economic loss associated with *Salmonella* (non-typhoidal serotypes only) and *E. coli* O157:H7, together in 2008, was estimated at \$3.2 billion (Economic Research Service/USDA, 2011). The preliminary FoodNet (Foodborne Diseases Active Surveillance Network) data for 2008 estimated the incidence of infections caused by foodborne pathogens like *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, Shiga toxin-producing *Escherichia coli* (STEC) O157, *Salmonella*, *Shigella*, *Vibrio*, and *Yersinia* did not change significantly as compared to the past three years (CDC, 2009). The lack of progress in the current food safety system demands further developing and evaluating of food safety control measures as food moves from farm to table.

For many years, *Salmonella* has been considered as the most important foodborne pathogen worldwide. Food categories including raw meat and poultry, eggs and egg products, milk and milk products, fresh produce and spices have been implicated in *Salmonella* outbreaks (de Roever, 1998). *Escherichia coli* strains are a common part of normal microbial flora of animals including human beings and are used as indicators of environmental fecal contamination of water supplies (Winfield and Groisman, 2003). Most of them are harmless, but some cause

diarrhea. Strains carrying particularly virulent properties have emerged as a serious hazard, with consumption of even low numbers of these microbes bearing life-threatening risks. During the last two decades, enterohemorrhagic *E. coli* (EHEC), producing verocytotoxins (VTs), also called Shiga-like toxins (SLTs), have emerged as a serious foodborne hazard (Cookson et al., 2007). Most of the initial human outbreaks are attributed to *E. coli* O157:H7 due to consumption of undercooked ground beef (Bell et al., 1994) and, occasionally, unpasteurized milk.

*Listeria monocytogenes* is a ubiquitous pathogen, which can infect at least 37 mammalian species, both domestic and wild, as well as at least seventeen species of birds and possibly some species of fish and shellfish. *L. monocytogenes* is remarkably a tough organism that can resist heat, salt, nitrite and acidity much better than many other organisms (Rocourt and Cossart, 1997). This bacterium can survive on cold surfaces and also multiply slowly at 24 °F, defeating traditional food safety defense (Refrigeration at 40 °F or below stops the multiplication of many other foodborne bacteria) (Rocourt and Cossart, 1997). Commercial freezer temperatures of 0 °F, however, will stop *L. monocytogenes* from multiplying. *Listeria* can cause serious and sometimes fatal infections in children, elderly people with a high mortality risk, infants, pregnant women, and the immune compromised (Gandhi and Chikindas, 2007). Foods commonly implicated in *Listeria* outbreaks often involve consumption of raw milk, butter, ready-to-eat meat products, surimi, smoked mussels and trout, and vegetables. In the United States, an estimated 2,797 persons become seriously ill, resulting in 500 deaths due to listeriosis each year (CDC, 2008). As per the Food safety and Inspection Service (FSIS) microbiological monitoring data from 1993-99, hot dogs/frankfurters and luncheon meats were the two major vehicles for foodborne contamination with *L. monocytogenes*. Center for Disease Control and Prevention has established an epidemiological link between consumption of hot dogs or undercooked chicken

with approximately 20% of the sporadic cases affected with listeriosis (CDC, 1992). Post-processing contamination, rather than failure of heating or pasteurizing processes, is usually suspected when *L. monocytogenes* is detected on processed products. In a survey conducted by Gombas et al. (2003) in the U.S. retail market (31,705 samples of various luncheon meats, seafoods, salads, and cheese), the incidence of *L. monocytogenes* was found in 1.82 % of the RTE foods sampled. This survey reported that eighty-two samples (0.89%; 9,199 of 31,705) were contaminated with *L. monocytogenes*. These numbers demonstrate that the incidences of *Listeria* have been a major threat to the food processing facilities and the consumers.

### **Justification**

Although several studies have been conducted to investigate different intervention strategies to reduce foodborne pathogen contamination, foodborne illness remains a major problem to the consumers and processors. To overcome this problem, investigating potent antimicrobials or intervention strategies using multiple hurdle technology that would reduce the pathogens to a minimal detectable level is required. Current trends in the food processing industry are focusing on reducing the extent or minimizing the use of conventional chemicals by replacing them with natural ingredients/extracts, and providing foods that require little or no preparation without post-processing contamination.

### **Multiple hurdle approach**

#### ***Conventional chemical antimicrobials***

Using multiple hurdle technologies could provide effective control measures in eliminating or reducing the impact of these pathogens on processors and consumers. Use of various chemical or natural antimicrobials to control foodborne pathogens in ready-to-eat meat and/or poultry products is encouraged by the interim final rule that was issued by the US

Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS 2006a) in response to various foodborne disease outbreaks associated with *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* Typhimurium (CDC, 2008, 2009). Conventional chemical antimicrobials, like lactates and diacetates as antimicrobial ingredients in formulations, are effective as safety hurdles in a multi-hurdle approach to ensure food safety in RTE processed meats. Including these antimicrobial chemicals in the formulation was found to be more effective than dipping in the antimicrobial solutions. Lactate and diacetate additives are effective antimicrobial agents for the control of *Listeria* and *Salmonella* when used in the formulations within their permissible levels. The maximum permissible levels of lactates and diacetates used in the meat formulations were 4.8 % and 0.25 % respectively by weight of the total formulation. These antimicrobial chemicals were found to have bacteriostatic activity more than bactericidal activity, and therefore may not be effective against gross contamination of a product (FSIS/USDA 2006b). Furthermore, higher concentrations of potassium lactate (> 3 %) and sodium diacetate (> 0.2 %) used in the formulation may affect the sensory qualities of the product, such as flavor and texture. Multiple lethality hurdles against *Listeria monocytogenes* can be provided by using lesser amount of these antimicrobial chemicals in combination with other natural antimicrobials.

#### ***Natural plant extracts – Antimicrobial activities***

Addition of natural plant extracts in food formulations as nutraceuticals is an increasing trend in the food industry. There is an increasing demand for safe, nutritious food products formulated with natural food ingredients. Plant extracts are being used in a variety of food applications to preserve food quality and enhance health benefits. Furthermore, the antimicrobial activities of several plant extracts including rosemary (Pszczola, 2002), grape seed and green tea extracts (Ahn et al., 2004; Rababah et al., 2005; Sivarooban *et al.*, 2007; Gadang et al., 2008;

Sivarrooban et al., 2008a, 2008b; Brannan et al., 2009; Over et al., 2009; Ganesh et al., 2010) have been demonstrated in broth as well as meat model systems. Rababah et al. (2005) reported that green tea extracts and grape seed extracts did not impart any undesirable color or flavor in poultry meat applications. Hence, including green tea and grape seed extracts in the hotdog formulations is expected to impart antimicrobial activity without affecting sensory properties. Finding potent plant extracts and food grade additives that can effectively enhance inhibition of foodborne pathogens is a continuing opportunity. Furthermore, there is limited knowledge on the antimicrobial activity of plant extracts incorporated in the hotdog formulations. Research is required to identify the most potent natural inhibitors of *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* Typhimurium that have caused foodborne illness and to select plant extracts that possess desirable antimicrobial and physicochemical properties to preserve product quality and to extend shelf life. The health image and the non-toxic nature of antimicrobials in plant extracts (grape seed and green tea extracts are used in a variety of food applications to preserve food quality as well as for nutraceutical and health benefits), and consumer perception and acceptability of products, demonstrate the demand and need for plant extract substitution to reduce conventional chemical antimicrobials in hotdogs.

### ***Post-processing heat treatment***

Food products may be contaminated during preparation i.e. post-process contamination. Foods that are pasteurized or cooked are sometimes subsequently exposed to the environment and may acquire pathogens before filling or packaging. RTE foods that have been subjected to a wide range of processing conditions, like storing, conveying, sorting, and packaging, create potential opportunities for contamination with foodborne pathogens. Pathogens such as *Salmonella*, *Listeria*, *E. coli*, and also some spore formers, can be introduced by a number of

vectors. Severe failure in one or more steps of processing, further processing, and handling may lead to contamination of the food product (Langfeldt et al., 1988; Steuer, 1992). Using post-process thermal treatments in addition to antimicrobials was found to be effective in decreasing the total microbial load. Metabolically injured pathogens by the antimicrobial treatments are susceptible to be killed by sub-lethal thermal treatment. FSIS compliance guidelines, to control *L. monocytogenes* in post-lethality exposed ready-to-eat meat products, pressure the ready-to-eat meat processing plants to include control programs to prevent *L. monocytogenes* (FSIS/USDA 2006b). The outcome of this study will facilitate the industries to adopt alternative 1 (application of post-lethality treatment and an antimicrobial agent or process to control *L. monocytogenes*) of the interim final rule for the control of *L. monocytogenes* (FSIS/USDA 2006c).

#### ***Physicochemical and sensory properties***

Addition of natural plant extracts may also enhance or provide a comprehensive protective system against lipid oxidation in the food product that would extend the shelf life. However, phenolic compound-rich, natural plant extracts like green tea and grape seed extracts may impart undesirable attributes such as color and bitterness to the final product if used in high concentrations. Therefore, hotdog formulations including chemical preservatives (organic acids salts – PL and SD) and plant extracts (GTE and GSE) combinations may affect the physicochemical (pH, water activity, color, and lipid oxidation). Further research is needed to investigate the optimum concentration levels of the natural plant extracts that would protect the food system against food contamination, lipid oxidation, while preserving the physicochemical and sensory properties.

Phenolic-rich plant extracts may contribute to the bitterness and astringency because of the interaction between phenolics, mainly procyanidins, and the glycoprotein in saliva, and thus

may influence sensory or organoleptic properties (appearance, juiciness, flavor, texture, and overall impression) (Dai and Mumper, 2010). Factors driving the consumer acceptance of any food product are color, moisture retention (juiciness), tenderness, and flavor (Jeremiah, 1978); this concept was further extended by subdividing to appearance and eating quality or palatability factors (Asghar and Pearson, 1980). Therefore, investigating the effect of natural plant extracts on the sensory properties (consumer acceptability) is needed.

### **Impact**

This study is directed towards establishing multiple hurdle technology by using reduced chemical preservatives, green tea, grape seed extracts and nisin in the hotdog formulations followed by post-process thermal treatments to control *L. monocytogenes*. This study is also targeted toward reducing chemical antimicrobials with natural “green” plant extracts and to provide the processing industry with a consumer attractive and preferred alternative of using natural sources of antimicrobials that are effective and inexpensive. This will provide a method to use natural antimicrobial “green” to control *L. monocytogenes* with reduced levels of chemical antimicrobials added to the hotdog during processing, but still have the same potential to kill/inhibit the growth of *L. monocytogenes*. There is no previous literature information available on the antimicrobial activity of grape seed and tea extracts and their combinations incorporated into hotdog formulations.

The overall goal of this his study is to use natural plant extracts (green tea and grape seed extracts), and to reduce the chemical antimicrobials such as lactates and diacetates used in RTE high and low fat chicken and turkey hotdogs to minimum level, and to inhibit the growth of *Listeria monocytogenes*. The objectives are:

1. Investigate the effect of conventional chemical preservatives used in hotdog on reducing or eliminating *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  colony forming

units per gram (cfu/g) levels in ready-to-eat high and low fat chicken and turkey hotdogs under normal and vacuum packaging during storage at 4 °C.

2. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA on inhibiting *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  cfu/g levels in ready-to-eat high and low fat chicken and turkey hotdogs under vacuum packaging during storage at 4 °C.
3. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA and heat treatment on inhibiting *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  cfu/g levels in ready-to-eat high and low fat chicken and turkey hotdogs under vacuum packaging during storage at 4 °C.
4. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA on physicochemical and sensory properties of ready-to-eat high and low fat chicken and turkey hotdogs during storage at 4 °C.

## REFERENCES

- Ahn, J., Grun, I.U. and Mustapha, A. 2004. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J Food Prot*, 67, 48–55.
- Asghar, A. and Pearson, A.M. 1980. Influence of ante- and postmortem treatments upon muscle composition and meat quality. In *Advances in Food Research*, 26, pp. 53-213, Academic Press, New York.
- Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., Tarr, P.L., Bartleson, C.A., Lewis, J.H. and Wells, J.G. 1994. A multistate outbreak of *Escherichia coli* O157:H7 associated bloody diarrhea and hemolytic syndrome from hamburgers: the Washington experience. *J Am Med Assoc*, 272, 149-1353.
- Brannan, R.G. 2009. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. *Meat Sci*, 81, 589–595.
- Centers for Disease Control and Prevention, 2011. Estimates of Foodborne Illness in the United States. Available from <http://www.cdc.gov/features/dsFoodborneEstimates/>, Accessed on January 17, 2012
- Centers for Disease Control and Prevention. 2009. Preliminary FoodNet Data on the Incidence of Infection with Pathogens Transmitted Commonly Through Food - 10 States, 2008. *Morbidity and Mortality Weekly Report*, 58, 333-337.
- Cookson, A.L., Bennett, J., Thomson-Carter, F. and Attwood, G.T. 2007. Intimin subtyping of *Escherichia coli*: Concomitant carriage of multiple intimin subtypes from forage-fed cattle and sheep. *FEMS Microbiol Lett*, 272, 163-171
- De Roever, C. 1998. Microbiological safety evaluations and recommendations on fresh produce. *Food Cont*, 9, 321-347.
- ERS/USDA (Economic Research Service, United State Department of Agriculture). 2011. Food-borne illness cost calculator.
- FSIS/USDA. 2006a. Food Safety and Inspection Service United State Department of Agriculture, *FSIS recalls archive*
- FSIS/USDA. 2006b. Food Safety and Inspection Service, United State Department of Agriculture Compliance guidelines to control *Listeria monocytogenes* in post-lethality exposed ready-to-eat meat and poultry products.
- FSIS/USDA 2006c. Verification procedures for consumer safety inspectors for the *Listeria monocytogenes* regulation and introduction of phase 2 of the *Listeria monocytogenes* risk-based verification testing program. *FSIS Directive*, 10, 240.4.

- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C.M. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *J Food Sci*, 73, 389–394.
- Gandhi, M. and Chikindas, M.L. 2007. *Listeria*: a foodborne pathogen that knows how to survive. *Int J Food Microbiol*, 113, 1–15.
- Ganesh, V., Hettiarachchy, N.S., Ravichandran, M., Johnson, M.G., Griffis, C.L., Martin, E.M., Meullenet, J.F. and Ricke, S.C. 2010. Electrostatic sprays of food-grade acids and plant extracts are more effective than conventional sprays in decontaminating *Salmonella* Typhimurium on spinach. *J Food Sci*, 75, 574–579.
- Gombas, D.E., Chen, Y., Clavero, R.S. and Scott, V.N. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. *J Food Prot*, 66, 559-569.
- Jeremiah, L.E. 1978. A review of factors affecting meat quality. *Lacombe Res. Stn. Tech. Bull. I*, Lacombe, Alb.
- Langfeldt, N., Heeschen, W. and Hahn, G. 1998. Zumm Vorkommen von *Salmonella* in Milchpulver: Untersuchungen zur Kontamination durch Analyse kritischer Punkte. *Kieler Milchwirtschaftl Forschungsber* 40, 81-90.
- Mead, P.S., Slutsker, L., Dietz, V., McCaiq, L.F., Bresse, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. 1999. Food-related illness and death in the United States. *Emerg Infect Diseases*, 5, 607-625
- Over, K. F., Hettiarachchy, N. S., Johnson, M. G. and Davis, B. 2009. Effect of organic acids and plant extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium in broth culture model and chicken meat systems. *J Food Sci*, 74, 515–521.
- Pszczola, D.E. 2002. Antimicrobials: setting up additional hurdles to ensure food safety. *Food Technol*. 56, 99-107.
- Rababah, T., Hettiarachchy, N.S., Eswaranandam, S., Meullenet, J.F. and Davis, B. 2005. Sensory evaluation of irradiated and nonirradiated poultry breast meat infused with plant extracts. *J Food Sci*, 70, 228–235.
- Rocourt, J. and Cossart, P. 1997. *Listeria monocytogenes*. In: Doyle, M.P., Beuchat, L.R., Montville, T.M. (Eds.), *Food Microbiology: Fundamentals and Frontiers*. American Society for Microbiology Press, Washington, DC, pp. 337–352.
- Sivaroban, T., Hettiarachchy, N.S. and Johnson, M.G. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10 °C. *J Food Prot*, 70, 1017–1020.

Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008a. Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extract. *J Food Prot*, 71, 2105–2109.

Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008b. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Res Int*, 41, 781–785.

Steuer, W. 1992. Hygienische Probleme bei der Teigwarenherstellung. *Mitt Gebeit Lebensmittelunters Hyg*, 83, 151-157.

Stone, H. and Sidel, J.L. 1993. Sensory evaluation practices (2nd ed.). San Diego: Academic Press.

Tauxe, R.V. 1997. Emerging foodborne diseases: an evolving public health challenge. *Emerg Infect Diseases*, 3, 425-434.

WHO (World Health Organization) 1997. Foodborne safety and Foodborne diseases, *World Health Stats Quaterly*, 50, 154 pp.

Winfield, M.D. and Groisman, E.A. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl Environ Microbiol*, 69, 3687-3694.

## CHAPTER II

### LITERATURE REVIEW

Each year, in the United States, foodborne illness causes forty-eight million cases of foodborne diseases, including 128,000 hospitalizations and 3,000 deaths (CDC, 2011). The economic losses associated with *Salmonella* (non-typhoidal serotypes only) and *E. coli* O157:H7 is estimated at more than \$3.0 billion by Economic Resource Service, USDA (ERS, 2007). Outbreaks of foodborne pathogens including *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* Typhimurium are of great concern to the food industry and the general public.

#### **Listeria monocytogenes**

The Genus *Listeria* is composed of *Listeria grayi*, *L. innocua*, *L. ivanovii*, *L. welshmeri*, *L. seeligeri*, and *L. monocytogenes*. All *Listeria* species are phenotypically similar, although, only *L. monocytogenes* and *L. ivanovii* are pathogenic (Rocourt and Buchrieser, 2007). *L. monocytogenes* is composed of twelve serotypes with 1/2b, 3b, 4b, and 4e of which genetic lineage I being most virulent (Rocourt and Buchrieser, 2007).

#### ***Growth characteristics***

*Listeria monocytogenes*, a gram positive, facultative anaerobe, psychrotroph, catalase positive, rod shaped bacterium (Farber and Perterkin, 1991) was named after “Lister” – a prominent taxonomist (Rocourt and Buchrieser, 2007). It was first isolated from diseased rabbits in 1926 and then later isolated from diseased animals with circling disease (neuromuscular incoordination), silage sickness, leukocytosis, cheese sickness, and tiger river disease (Murry et al., 1926). *Listeria* is the causative agent of “*listeriosis*,” a severe disease with high hospitalization and case fatality rates. It is a hardy bacterium, which can grow across a broad range of pH (4.3 – 9.8) (Seelinger and Jones, 1986) and temperature (0.5 - 45 °C) (Ralovich, 1992). The favorable pH for *listeria* growth in meat products is generally at pH 6.0, and grows

poorly, or not at all, below pH 5.0 (Glass and Doyle, 1989). It can also grow in the presence of high salt concentrations (up to 20% w/v; osmotolerant) and low water activity ( $a_w$ , 0.91) (Lado and Yousef, 2007). The ability to grow at low temperatures allows this pathogen to overcome food safety measures and pose a serious risk to human health (Smith, 1996; Gandhi and Chikindas, 2007). Its survival and growth at refrigerated temperatures (2 - 4 °C) make this pathogen difficult to control (Rocourt and Cossart, 1997). This can be attributed to its ability in making changes to membrane fatty acid composition (Beales, 2004), production of cold shock proteins (Bayles et al., 1996), and accumulation of compatible solutes, such as glycine betaine and carnitine (Angelidis and Smith, 2003), in response to the low temperatures.

### ***Epidemiology***

*Listeria monocytogenes* can be found in a wide variety of raw and processed food products such as milk and dairy products, various meat and poultry products such as fermented sausages, deli meats, frankfurters and fresh produce such as radishes, cabbage, and sea food and fish products (Rocourt and Cossart, 1997). It contaminates the products mainly after thermal processing, thus it is a post-processing contaminant in the food establishments. Factors that contribute to the persistence in the food processing environments are its growth at low temperature, biofilm forming abilities, (Di Bonaventura et al., 2008) and sanitizing resistance (Lunden et al., 2003). Listeriosis has been associated with RTE processed foods and meats since the recognition of *Listeria* as a foodborne pathogen in the 1980s due to consumption of non-reheated frankfurters (Schwartz et al., 1988). The first outbreak in US associated with *Listeria* was reported in 1979 with raw vegetables (Ho et al., 1986). The common food implicated in *Listeria* contamination are RTE deli meats, soft cheese, hotdogs, and sea food due to the intrinsic properties of the food (high protein, moderate water activity, low back ground microflora) and

the environmental niches that are favorable for *Listeria* growth (Swaminathan and Gerner-Smidt, 2007).

### ***Pathogenesis***

The infective dose required to cause illness in susceptible individuals can be in the order of 100 – 1000 cells even in non-immune compromised subpopulations (FSIS/USDA, 2003; Drevets and Bronze, 2008). Below 100 cfu/g, the chance of infection is very small even in chronically exposed, vulnerable individuals (Buchanan et al., 1997; Chen et al., 2004). Consumption of food products contaminated with a higher dose ( $> 8 \log$  cfu) of *L. monocytogenes* can cause gastroenteritis in healthy individuals (Warriner et al., 2009). Once ingested, *Listeria* can invade the gastrointestinal epithelium and become bloodborne where the bacterium becomes associated with monocytes then subsequently the liver, spleen and lymphatic system (Drevets and Bronze, 2008).

### ***Control measures***

According to the US Food and Drug Administration (FDA), presence of *L. monocytogenes* on a ready-to-eat food or food contact surface is classified as an adulterant, and triggers a product recall (zero-tolerance policy). The specific final rule of the FDA was found in Federal Register Interim Final Rule 9 CFR Part 430 (Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products, 2006).

1. “*L. monocytogenes* can contaminate RTE products that are exposed to the environment after a lethality treatment (destroy/kill).”
2. “*L. monocytogenes* is a hazard that an establishment must control through its HACCP plan, or prevent in the environment through a Standard Sanitation Operating Procedures (SSOPs) or other prerequisite program if it produces RTE product that is exposed post-lethality.”

3. “RTE product is adulterated if it contains *L. monocytogenes* or if it contacts surfaces contaminated with *L. monocytogenes*.”

The final rule published by the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) in October 2003 has established three alternative means for regulating RTE meat and poultry products that are exposed to the environment after cooking (USDA/F SIS, 2003). To comply with the regulations, food processors are provided with three alternatives,

- “For high risk foods such as RTE meat products, **Alternative 1** is preferred which combines a post-lethal decontamination step along with formulation hurdles.”
- “For moderate risk foods, **Alternative 2** is preferred with the option of applying a post-lethal decontamination step or formulation hurdles along with *Listeria* sanitation program.”
- “Finally, for low risk foods like vegetables and salads, **Alternative 3** relies on having an effective sanitation program along with end-product testing. However, if used for RTE meats, a hold and release policy must be enforced.”

## **HOTDOGS/FRANKFURTERS**

Hotdogs represent convenience RTE foods that are popular in the U.S. markets. In 2010, consumers spent more than \$1.6 billion on hotdogs and sausages in U.S. supermarkets (National Hotdog and Sausage Council, 2011).

### ***Hotdogs – Source of contamination and recalls***

Hotdogs are often consumed without further heating and thus require stringent control measures during processing. In general, contamination of hotdogs occurs after processing or just before consumption. In the last three years (2007 – 2009), five Class-I frankfurter recalls involving 47,864 lbs., not including other ready-to-eat (RTE) meats and sausages, have been

reported for contamination with *L. monocytogenes* (USDA/FSIS, 2007, 2008, 2009). According to FSIS, the recommended storage of frankfurters at 40 °F (4.4 °C) in opened packages is not more than seven days or fourteen days in vacuum-packages (FSIS, 2006).

## **CHEMICAL COMPOSITION OF CHICKEN AND TURKEY MEAT**

Two common muscle food categories that are widely popular, and more consumed in poultry, are broiler and turkey meat. In both broiler and turkey carcasses, breast and thigh (leg) portions are the common meat portions. However, the nutrient (protein and lipids) content varies from one portion to another within the same carcass, as well as between chicken and turkey.

In broilers, moisture content is higher in breast meat than thigh muscle (73.74 vs. 73.22 %) though it is not significantly different (Gornowicz and Dziadek, 2001) and varies depending on the carcass portion. The moisture content of broiler meat (74.36 %) is higher than turkey (72.74 %) (Qiao et al., 2002; Filus et al., 1995). The protein richest portions are the breast meat of turkeys followed by broilers, compared to thigh muscles of all types of poultry, while the fat content in the breast meat is lower than thigh meat across all poultry meat (Lesiow, 2006).

Lipid fraction of the meat plays an important role in nutritional and sensory properties of the meat product. Furthermore, it also has a direct effect on the flavor and serves as a solvent and precursor of aroma compounds. Fat present in the food products contributes to taste sensation by the release of desirable flavors in the mouth, affecting retention of flavor components in the mouth, or affecting the interaction between flavor compounds and flavor receptors on the tongue (Kinsella, 1990).

Deposition of lipids in the muscle may be intramuscular or intermuscular. Intramuscular lipids refer to lipids contained in both intramuscular adipose tissue and muscular fibers and flavor intensity increases with increase in intramuscular fat (Fostier et al., 1993). Intramuscular

adipose tissue consists of fat cells rich in triacylglycerols (TAG) located along the muscle fibers as well as interfascicular area. Triacylglycerols content is lowest in broiler breast (0.24g/100g) and average in turkey (0.54g/100g) among poultry (Sklan et al., 1983, 1984). The phospholipids (PL) content in breast muscles was lower than thigh muscles in both chicken and turkey meat. However, broiler muscle lipid fraction contained more free fatty acid (FFA) amounts than turkey muscles. The fatty acid composition of muscle tissue plays a key role in lipid stability and product quality. The higher the polyunsaturated fatty acid (PUFA) content in the meat, the more chances of deteriorative changes due to lipid oxidation. In general, the PUFA content was higher in turkey than broiler carcasses (Komprda et al., 2002). Differences in the chemical composition of chicken and turkey meat are highlighted in Table 2.1.

**Table 2.1** Chemical composition of chicken and turkey meat

<b>Parameter</b>	<b>Chicken</b>	<b>Turkey</b>
<b>Moisture (%)</b>	74.36	73.51
<b>Protein (%)</b>	22.39	19.54
<b>Total lipid (%)</b>	1.48	4.84
<b>Total lipids (g/100 g)</b>	0.97	2.03
<b>Triacyl glycerols (TAG) g/100 g</b>	0.35	1.14
<b>Phospholipids (g/100 g)</b>	0.68	0.76
<b>Free Fatty Acids (g/100 g)</b>	0.02	0.02
<b>FATTY ACID PROFILE</b>		
<b>Arachidonic acid (AA, C20:4n6)</b>	3.65	3.27
<b>Eicosapentaenoic acid (EPA, C20:5n3)</b>	0.62	0.03
<b>docosahexaenoic acid (DHA, C22:6n3)</b>	2.95	0.18
<b>Linoleic (LA, C18:2n6)</b>	13.38	15.36
<b>Linolenic (LNA, C18:3n3)</b>	0.52	1.99
<b>Saturated fats (SFA)</b>	39.46	31.79
<b>Mono-unsaturated fats (MUFA)</b>	29.62	43.4
<b>Polyunsaturated fats (PUFA)</b>	23.19	22.38
<b>VITAMINS</b>		
<b>B1-Thiamine (mg/100g)</b>	0.07	0.08
<b>B2-Riboflavin (mg/100g)</b>	0.09	0.22
<b>Niacin (PP) (mg/100g)</b>	11.19	3.07
<b>Pyrdoxine (B6) (mg/100 g)</b>	0.55	0.36
<b>Vit- E (mg/100g)</b>	0.50	0.14

Lesiow, T., 2006.

## **CONTROL MEASURES TO REDUCE FOODBORNE PATHOGENS**

### **Food preservation**

Food preservation is one of the oldest technologies used by human beings. Historically, boiling, freezing and refrigeration, salting, curing, drying, pasteurizing, dehydrating, pickling and packing are the methods used to preserve food products (Leistner, 2000). Among the earliest preservatives are sugar and salt (NaCl), which produced food environments of high osmotic pressure that inhibited the growth of bacteria in the aqueous surroundings (Shee et al., 2010). For example, jams and jellies are preserved as solutions of high sugar content, and many meats (e.g. hams) and fish are still preserved by salting (Harris, 2007).

### **Chemical preservatives**

Chemical preservatives are effective for longer shelf life and are generally fool proof for preservation purposes because of their effectiveness in various food matrices and environmental conditions (Shee et al., 2010).

- Benzoates (E.g. sodium benzoate, benzoic acid)
- Sulphites (E.g. sulphur dioxide),
- Sorbates (E.g. sodium sorbate, potassium sorbate)
- Acetates and lactates (potassium or sodium lactate and sodium diacetate) (Shelef, 1994)

The use of various chemical, or natural antimicrobials, to control foodborne pathogens in RTE meat and/or poultry products is encouraged by the interim final rule that was issued by US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) in response to various foodborne disease outbreaks associated with *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* Typhimurium (CDC, 1999, 2000, 2002).

### ***Lactates and diacetates***

Lactates and diacetates are the most common chemical antimicrobial agents, used alone or in combination, in meat and poultry products to control *L. monocytogenes* (Tompkin, 2002). Both potassium lactate (PL) and sodium diacetate (SD) have been approved by USFDA (FDA, 2000), and combinations of these two can effectively inhibit the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage (Mbandi and Shelef 2001; Seman et al., 2002; Sommers et al., 2003 and Barmpalia et al., 2004).

Potassium lactate ( $\text{CH}_3\text{CHOHCOOK}$ , mol. wt. = 128.18) is a salt of lactic acid available commercially as 60% neutral aqueous solution. It is considered a Generally Recognized As Safe (GRAS) food ingredient used as humectant, a flavor enhancer, and bacteriostatic agent at levels up to 4.8% in emulsified products (CFR, 2002). Specific actions of lactate can be explained in three mechanisms: (1) reducing the water activity ( $a_w$ ) of the product (Brewer et al., 1991; Chen and Shelef 1992; Miller and Acuff; 1994), (2) intracellular acidification (Hunter and Segal, 1973), and (3) antilisterial activities of lactate ions (Maas et al., 1989). Reducing the pH (increasing the acidity) of the meat product may cause less purge in the packed product (Nunez et al., 2004). Inclusion of lactates as formulatory ingredient or antimicrobial dip would not have any effect in color (surface and internal) and sensory attributes in frankfurters (Nunez et al., 2004).

Lactates of sodium or potassium have demonstrated the antimicrobial effects in various meat products (Papadopoulos et al., 1991; Bradford et al., 1993; Miller and Acuff 1994; Shelef 1994; Brewer et al., 1995; Nerbrink et al., 1999; Stekelenburg and Kant-Muermans 2001; Glass et al., 2002; Porto et al., 2002; Samelis et al., 2002; Tan and Shelef 2002; Choi and Chin 2003). Addition of potassium lactate slightly increased fatty flavor/aroma, astringent and bitter tastes,

and bitter aftertaste attributes in the meat products (Bradford et al., 1993; Nunez et al., 2004). In general, lactate sensitivity is higher in Gram-positive than Gram-negative bacteria under optimum growth conditions (6.5 pH, 20 °C) (Houstma et al., 1993).

Sodium diacetate ( $C_4H_7O_4Na \cdot xH_2O$ , Mol. wt. = 142.09) is a mixture of acetic acid, sodium acetate, and water of dehydration, and is a GRAS chemical compound that can be effectively used to control foodborne pathogens in various meat and poultry products within the permissible level (0.25% of total weight of the formulation; 21 CFR 184.1754). It can be also used as a flavoring agent, adjuvant, and pH control agent. As a sodium acidulant, it can reduce the pH of the system in which it is added (Mbandi and Shelef, 2002). It can be used alone or in combination to inhibit foodborne pathogens in meat products such as frankfurter, deli meats, and bologna (Barmapalia et al., 2005). The minimum inhibitory concentrations (MIC) of sodium diacetate in brain heart infusion (BHI) broth were determined to be 35 mM, 32 mM, and 28 mM at 35°C, 20°C, and 5°C, respectively (Shelef and Addala, 1994). Based on equal levels of undissociated acetic acid at different pH values, sodium diacetate was more effective and had lower minimum inhibitory concentrations at 35°C than did the acetic acid (Shelef and Addala, 1994). Sodium diacetate was also effective against *Pseudomonas fluorescens*, hemorrhagic *E. coli*, *S. Enteritidis*, *Shewanella putrefaciens*, and *B. cereus* and with no effect against *Pseudomonas fragi*, *Y. enterocolitica*, *Enterococcus faecalis*, *Lactobacillus fermentans*, or *S. aureus* (Shelef and Addala, 1994).

Effective use of lactates and diacetates also depends on the dissociation constant (pKa) or the pH at which 50% of the total acid is dissociated. As most of the organic acids have their pKa between pH 3 – 5 range, it would be effective to use the acidulants or organic acids as antimicrobials in food system with pH beyond their pKa range. Organic acids having less than

seven carbons were more effective at lower pH, and acids with eight to twelve carbons were more effective at neutrality and above (Hoffman et al., 1939). Lactates and diacetates in various combinations within in the permissible levels can be effectively used as additional hurdles to control the foodborne pathogens. Sodium lactate at 2-3 % levels and sodium diacetate at 0.1 – 0.3 % levels were equally effective as antilisterial compounds in meat products with little effect on pH and sensory characteristics (Mbandi and Shelef, 2001). The most effective combination of lactates and diacetates that are currently used in the meat industry is 2 % sodium (or) potassium lactate and 0.1-0.15 % sodium diacetate (Barmpalia et al., 2005; Stekelenburg, 2001).

## **PLANT EXTRACTS AS ANTIMICROBIAL AGENTS**

Utilization of plant extracts, as an alternative to conventional chemical or synthetic antimicrobials to combat foodborne pathogens and extend shelf life, is an increasing trend in the food industry. Major groups of chemicals present in plant extracts include polyphenols, quinones, flavanols/flavanoids, alkaloids, and lectins (Cowan, 1999). Phenolic extracts prepared from sage, rosemary, thyme, hops, coriander, green tea, grape seed, cloves, and basil are known to have antimicrobial effects against foodborne pathogens (Shelef, 1984; Juven et al., 1994; Larson et al., 1996; Davidson and Naidu, 2000; Elgayyar et al., 2001; Hong et al., 2009; Bisha et al., 2010). Natural plant extracts used along with other hurdles like low storage temperature, low pH, anaerobic conditions, organic acids, bacteriocins, and irradiation showed synergistic antimicrobial action in various food systems (Beuchat et al., 1994; Gadang et al., 2008; Over et al., 2009).

### ***Green tea and grape seed extracts***

Finding potent plant extracts that can be used as food grade additives, which can effectively inhibit foodborne pathogens and/or have antioxidant properties, is a continuing

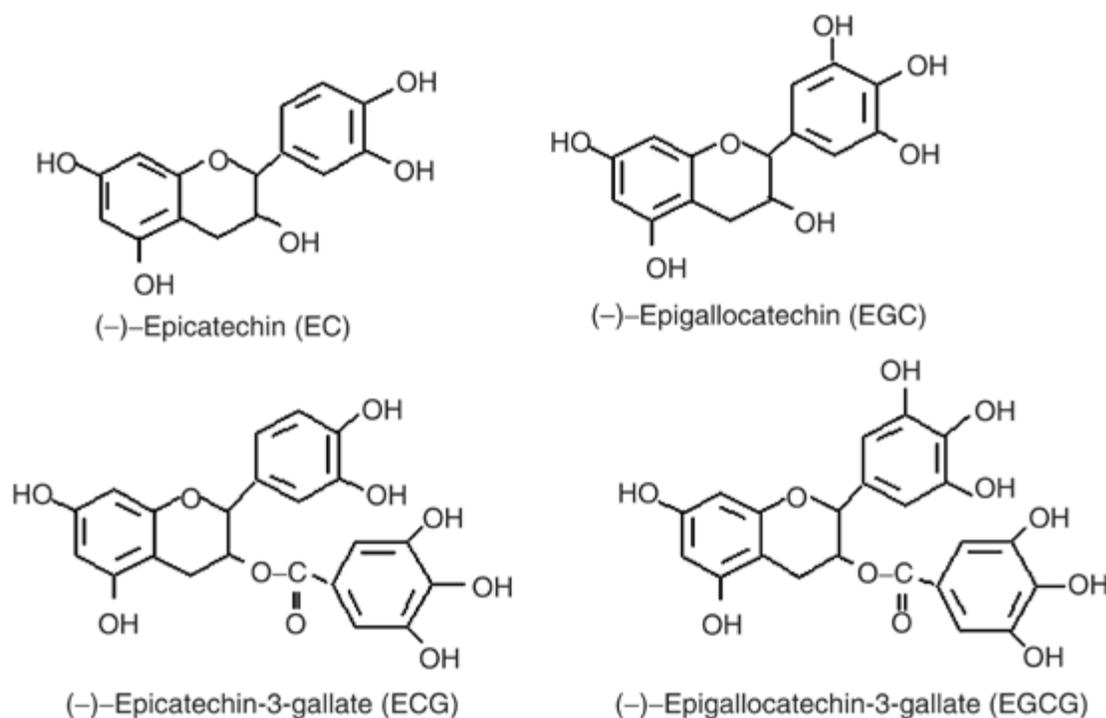
challenge and opportunity. Green tea and grapes are traditional, popular beverages that have diverse health benefits including antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties (Xia et al., 2010). Multiple lethality hurdles to the growth of pathogens including *Listeria monocytogenes*, *E. coli* O157:H7, and *Salmonella* Typhimurium can be provided by using lesser amount of conventional chemical antimicrobials in combination with natural plant extracts such as green tea and grape seed extracts (Gadang et al., 2008). Reducing conventional chemical antimicrobials and incorporating natural “green” plant extracts will provide the processing industry with a consumer attractive and cost effective alternative. Furthermore, the health image and non-toxic nature of antimicrobials in plant extracts, as well as consumer perception and acceptability of products containing grape seed and tea extracts, has been well demonstrated (Ahn et al., 2004; Bisha et al., 2010).

### ***Green tea extract (GTE)***

Tea (black, green, white and oolong) is a widely consumed beverage that has attracted considerable attention in recent years due to numerous health benefits such as antioxidant, antimicrobial, anti-carcinogenic and anti-arteriosclerotic properties (Matthews, 2010; Cooper et al., 2005). Green tea extract is a derivative of cultivated evergreen tea plant (*Camellia sinensis* L.) of the family Theaceae, processed by spray drying the strong infusions after they have been concentrated to solids (40 – 50 %) (Wang et al., 2000). Fermentation and heating processes yield polymerization of catechins (mono polyphenols) and conformational changes, which ultimately contribute to various properties of tea. Based on the degree of fermentation during manufacturing process, tea can be classified into three major types: non-fermented-green tea (~20 %), fermented-black tea (~78%), and the semi fermented-oolong tea (~2%) (Cheng and Chen, 1994; Wei et al., 2009).

### ***Green tea extract - chemical constituents***

The chemical composition of tea is complex, consisting of polyphenols (catechins and flavonoids), alkaloids (caffeine, theobromine, theophylline), volatile oils, polysaccharides, aminoacids, lipids, vitamin C, minerals, and other uncharacterized compounds (Karori et al., 2007). Among these, the largest component present in green tea leaves is carbohydrates (including cellulosic fiber) and the simplest compounds are catechins, a group of flavanoids called flavan-3-ols (Yilmaz, 2006). These catechins are synthesized in tea leaves through malonic acid and shikimic acid metabolic pathways with gallic acid as an intermediate derivative (Naidu, 2000). Catechins are colorless water-soluble compounds that impart bitterness and astringency to green tea infusions (Wang et al., 2000). Total catechin content in green tea is 420 mg/L (Auger et al., 2004). Catechins constitute 15-30% of the dry weight of green tea leaves as opposed to 8-20% of oolong and 3-10% of black tea (Amidor, 2009). Green tea extract contains six primary catechins, namely, (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) (Kajiya et al, 2004). EGCG is the most important and well-studied tea catechin due to its high content (as high as 50 %) in tea and has the most potent physiological properties while (+) –GC and (+) –C are usually present in trace components (Stewart et al., 2004; Taylor et al., 2005). Ester type catechins, (-)-ECG and (-)-EGCG are stronger in bitterness and more astringent than (-)-EC and (-)-EGC and these flavanoids have synergistic action unlike individual tea components (Fujiki, 1999; Han and Chen, 1995). It is estimated that a cup of green tea (2.5 g of green tea leaves/200 ml of water) may contain 90 mg of ECG (Wu and Wei, 2002). These flavonoids may be found in a cup of tea at 1 mg/ml concentration level (Sakanka et al., 1989).



**Figure 2.1** Structure of major tea catechins present in green tea extract

Adapted from Bigelow, R.L.H. and Cardelli, J.A. 2006. The green tea catechins, (-)-Epigallocatechin-3-gallate (EGCG) and (-)-Epicatechin-3-gallate (ECG), inhibit HGF/Met signaling in immortalized and tumorigenic breast epithelial cells *Oncogene* 25, 1922–1930 (23 March 2006) | doi:10.1038/sj.onc.1209227).

### ***Antimicrobial activities – Mechanism of action***

Polyphenols extracted from green tea extract have shown inhibitory effects on Gram-positive as well as Gram-negative bacteria (Gadang et al., 2008). The susceptibility of GTE against various bacterial strains can be related to differences in cell membranes (Ikigai et al., 1993). Yoda et al. (2004) investigated the antibacterial activities of EGCG on various strains of *Staphylococcus* (Gram-positive cocci) and Gram-negative rods including *E. coli*, *Klebsiella pneumoniae*, and *Salmonella* and found that 50-100 µg/mL was required to inhibit growth of

*Staphylococcus*, and concentrations higher than 800 µg/mL was required to inhibit Gram-negative rods.

Among the catechins present in green tea extract, EGCG and ECG are the most potent in exhibiting antimicrobial activity due to the galloyl moiety present in their structures (Fig 2.1) (Shimamura et al., 2007). The outer cell membrane, or cytoplasmic membrane of a bacterium, is essentially composed of a phospholipid bilayer and proteins and is the major site of interaction with antimicrobial compounds. Damage to this vital membrane can result in death of the bacterium and can occur in the following ways: (i) physical disruption of the membrane (Shimamura et al., 2007), (ii) dissipation of the proton motive force (PMF) (Juven et al., 1994), and (iii) inhibition of membrane-associated enzyme activity.

Functional hydroxyl groups and conjugated double bonds in the reactive groups of natural plant extracts may be involved in their binding to the cell wall components (usually proteins) (Masson and Wasserman, 1987). Catechins (galloyl and gallic moieties) have a deteriorating effect on the lipid bi-layer membrane resulting in loss of cell structure and function, eventually leading to cell death (Ikigai et al., 1993; Tsuchiya et al., 1996; Cox et al., 2001). Presence of gallic acid esters in EC, EGCG are responsible for their high affinity for lipid bilayers, and affect the membrane structure (Hashimoto et al., 1999). Major phenolic constituents like epicatechin, caffeic acid, benzoic acid, and syringic acid may alter the cell morphology by influencing the osmotic pressure of the cell, thus disrupting the cytoplasmic membrane and causing leakage of cell constituents (Davidson and Naidu, 2001; Sivarooban et al., 2008a).

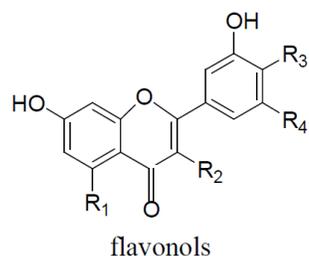
### ***Grape Seed Extract (GSE)***

Grape seed extract is a by-product derived from grape seeds (*Vitis vinifera*) (from grape juice and wine processing) that is extracted, dried and purified to produce a polyphenolic

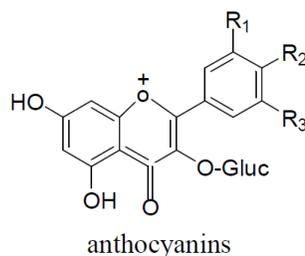
compound-rich extract (Lau and King, 2003). Grape seed extract is sold commercially as a dietary supplement listed on the “Everything Added to Food in the United States (EAFUS)” and also has Generally Recognized as Safe (GRAS) status approved by Food and Drug Administration (FDA). However, few studies have reported the side effects of GSE when used alone or in combination with other supplements such as antiplatelet properties, interactions with other medicines and supplements (Shanmuganayagam et al., 2002; Rein et al., 2000). This may be effective effective when GSE is used at pharmacological doses (150-300 mg/day) (Clouatre and Kandaswami, 2005) whereas in the food applications it is used at fairly low concentrations (0.01 - 1%). Furthermore, the no-observed-adverse effect level (NOAEL) of GSE determined in rats was 1.78g/kg of body weight/day, which was evidently a higher concentration level than we normally use in food applications (Bentivegna and Whitney, 2002).

#### ***Chemistry of grape seed extract - principal components***

Standardized grape seed extracts contain 74 to 78% oligomeric proanthocyanidins and less than approximately 6% of free flavanol monomers on a dry weight basis (Burdock, 2005). Proanthocyanidins in the form of monomeric phenolic compounds, such as catechin, epicatechin and epicatechin-3-O-gallate, and in dimeric, trimeric, and tetrameric procyanidin forms are rich in GSE. These can combine with gallic acid to form gallate esters and ultimately glycosides (Fig 2.2) (Negro et al., 2003; Weber et al., 2007). The red color and astringency taste of the GSE can be attributed to polyphenol-rich compounds, especially proanthocyanidins, which may affect the color and sensory characteristics of the product when used at higher concentrations (Monteleone et al., 2004; Weber et al., 2007).



	<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>	<b>R<sub>4</sub></b>
quercetin	OH	OH	OH	-
rutin	OH	O-Rutinose	OH	-
morin	OH	OH	-	OH
myricetin	OH	OH	OH	OH
fisetin	OH	OH	OH	-



	<b>R<sub>1</sub></b>	<b>R<sub>2</sub></b>	<b>R<sub>3</sub></b>
peonidin-3- <i>O</i> -glucoside	OCH <sub>3</sub>	OH	-
petunidin-3- <i>O</i> -glucoside	OH	OH	OCH <sub>3</sub>
malvidin-3- <i>O</i> -glucoside	OCH <sub>3</sub>	OH	OCH <sub>3</sub>
cyaniding-3- <i>O</i> -glucoside	OH	OH	-
delphinidin-3- <i>O</i> -glucoside	OH	OH	OH

**Fig. 2.2** Chemical structures of some phenolic compounds from grape seeds.

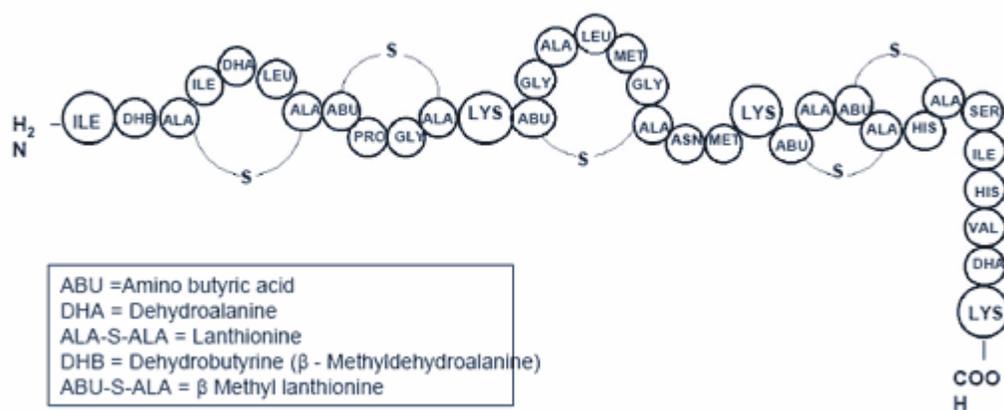
Adapted from Xia et al. (2010). Biological activities of polyphenols from grapes — Review. *International Journal of Molecular Sciences*, 11, 622–646.

*Antimicrobial properties – mechanism of action*

The core structures with 3, 4, 5-trihydroxyphenyl groups found in epigallocatechin, epigallocatechin-3-Ogallate, castalagin, and prodelphinidin might be important for antibacterial activity (Tagurt et al., 2004). As GSE is a rich source of polymers of flavon-3-ols like (+) - catechin and (-) -epicatechin), its antimicrobial properties can be attributed to general

mechanisms of phenolics as discussed earlier with the green tea extract. The red-pigmented polymeric phenolics from juice and skin showed pH-dependent antilisterial activity, while the unpigmented polymeric phenolics demonstrated antilisterial activity that was independent of pH (Rhodes et al., 2006). The increasing order of the antimicrobial activity reported in grape plant was flesh, whole fruit grape extracts, fermented pomace, skin, leaves, and seeds (Xia et al., 2010). Anastasiadi et al. (2009) suggested that high concentrations of flavonoids and their derivatives in grape seeds and flavonoids, stilbenes, and phenolic acids in grape stems were responsible for the antimicrobial activity. Vaquero et al. (2007) concluded that the non-flavonoid caffeic acid and the flavonoids such as rutin and quercetin had higher inhibitory activities on the growth of *Listeria monocytogenes*. Rhodes et al. (2006) demonstrated that polymeric phenolic fractions produced the highest inhibition activity for all *Listeria* species, but not for other bacteria, such as *Bacillus cereus*, *Salmonella Menston*, *Escherichia coli*, *Staphylococcus aureus*, or *Yersinia enterocolitica*.

## NISIN



**Fig 2.3** Structure and chemical composition of nisin

Nisin is a 34 amino acid bacteriocin (antimicrobial peptides produced by bacteria) produced by *Lactococcus lactis* strains during its exponential phase of growth (Buchaman et al., 2000) and is approved (GRAS) in the U.S. (21 CFR 184.1538). Nisin belongs to the class of 'lantibiotics' due to the presence of unusual amino acid lanthionine (Fig 2.3). Nisin was first discovered in the late 1920s and early 1930s when it was first described as a toxic substance in the milk that adversely affected the starter cultures (Rogers and Whittier, 1928; Whitehead, 1930). It is widely used as a preservative in dairy and meat industries to control pathogens such as *L. monocytogenes*. Control of foodborne pathogens by nisin can be delivered in two approaches: (1) Addition of purified or partially purified bacteriocin directly to the food as an ingredient. (2) Addition of bacteriocin producing bacteria to the food product in in situ form. Nisin permeabilizes the cytoplasmic membrane of the target cells, forming pores to leak out the cytoplasmic substances from the cell (Abee et al., 1994). As nisin and phenolics-rich plant extracts, like grape seed and green tea, have common mode of action i.e., action on bacterial cytoplasmic membrane, the combination of such compounds is expected to have additive inhibition or synergistic action on various pathogens (Theivendran et al., 2006).

### ***Mode of action***

Nisin initially forms a complex with lipid II (precursor molecule in bacterial cell wall synthesis), then inserts into the cytoplasmic membrane forming pores to leach out the essential cellular components, which ultimately leads to inhibition or death of bacteria. The antimicrobial effect of nisin depends on concentration of bacteria and pH in the system, thus its antimicrobial effect decreases with high bacterial load and pH (Broughton, 2005; Theivendran et al., 2006). The method of delivery of bacteriocins in an encapsulated form into the food systems for improving its stability and antimicrobial action is very promising. Quick release of encapsulated

nisin into the food system would provide short-term antimicrobial effects, whereas the immobilized nisin would provide an inhibitory effect over a long period due to its slower desorption from the membrane (Benech et al., 2002). The stability of nisin in the food system during storage is dependent upon three factors: incubation temperature, length of storage, and pH. At low temperatures, the retention of nisin increases.

### ***Antimicrobial properties of nisin***

Though nisin is effective against spores and gram-positive bacteria such as *L. monocytogenes*, it does not have a significant inhibitory effect on gram-negative organisms, yeasts or moulds (Boziaris and Adams, 1999, Cannarsi et al., 2008). This resistance is due to the protective outer membrane that forms the outer most layer of the cell envelope, as an effective barrier against hydrophobic solutes and macromolecules (Hurst, 1981; Hauben et al., 1996; Broughton, 2005) However, it can be effectively used when combined with agents that make the cell walls permeable to nisin, such as chelating agents like ethylenediaminetetraacetic acid (EDTA), sub-lethal heat, osmotic shock, and freezing (Boziaris and Adams, 1999). At low levels, EDTA can enhance the nisin activity against *L. monocytogenes* (Branen and Davidson, 2004). Lactoferrin in combination with 50% less nisin inhibited *L. monocytogenes*, while lactoferrin alone did not show any bacteriostatic effect. Nisin also showed synergistic action with plant extracts like thymol towards inhibition of *L. monocytogenes* and *B. subtilis* (Helander et al., 1998; Ettayebi et al., 2000). Thymol alters the bacterial membrane structure (outer membrane disintegration) resulting in greater permeability for nisin, thus permitting lower nisin concentration to obtain the same level of antibacterial activity (Ettayebi et al., 2000).

### ***Nisin – use in multiple hurdle technology approach***

Nisin has also proven to be very useful as a part of hurdle technology, where a combination of two or more treatments is used to obtain a more effective method of food preservation (Cleveland et al., 2001). Nisin can be used as a formulatory ingredient, antimicrobial dips alone, or in combination with other antimicrobials to provide additional hurdle and margin of safety to the food products. Nisin, along with carbon dioxide, has a synergistic action on wild type *L. monocytogenes* to give a four-log reduction in cell count with no effect on nisin-resistant cells grown in the presence of air or carbon dioxide (Nilsson et al., 2000). The presence of carbon dioxide increases the permeability and the proportion of short-chain fatty acids in the cell membrane, which helps in the pore formation by nisin (Nilsson et al., 2000). Nisin also has synergistic action when combined with thermal treatments, non-thermal treatments like Pulsified Electric Fields (PEF) in inactivation *Listeria* spp. in food products (Modi et al., 2000; Calderon-Miranda et al., 1999a, b). Application of nisin at 1.25-6.25 mg/kg or dipping the cooked sausage into nisin solutions (5-25 mg/L) increased shelf life at 6-12 °C (Broughton, 2005).

## **POST-PROCESSING INTERVENTION STRATEGIES**

Even though the RTE food products will be free of pathogens through the cooking process, there could be a surface contamination of the food products before packaging. Therefore, it will be beneficial to decontaminate the outer layer of the food products by post-processing intervention treatments such as thermal treatments, irradiation, and high-pressure exposure.

### ***Thermal treatments***

Post-process interventions, either alone or in combination with antimicrobial compounds, can be used to inhibit the proliferation of foodborne pathogens. Flash pasteurization can be used to decontaminate the surfaces of fine emulsified sausages such as frankfurters before packaging

(Murphy et al., 2005a, 2005b, 2006). Post-process intervention strategies that provide moderate action on pathogens when used in combination with the antimicrobials (chemical or natural) have the potential to significantly reduce the frequency of frankfurter recalls and foodborne illness outbreaks.

D-value (thermal decimal reduction time) is the time required to reduce the microbial/spoilage population by 90% (1 log unit) in a well-defined medium, and it is an indicator of thermal resistance of a microorganism at a constant temperature (Montville and Matthews, 2005). The thermal resistance (*D*- value) of bacteria depends on age of the culture, stage of growth cycle and growth conditions, temperature, food characteristics such as salt concentration, water activity, acidity, and exposure to stress conditions like sub lethal heat shock. *Listeria* is more heat resistant than other non-spore formers such as *Salmonella* and *E. coli* (Beauchemin, 1990; Rybka-Rodgers, 2001). The z-value is the temperature increment required to reduce the *D*-value by 90%, and it is an indicator of the temperature dependence of microbial inactivation. A comprehensive knowledge of “D” and “z” values of particular pathogens will provide processors sufficient information to design thermal treatments (Stumbo, 1973 and Pflug, 1997). The highest D-value measured for *L. monocytogenes* in food at 70 °C was 0.27 min, while z-values ranged from 5.98 to 7.39 (Gaze et al., 1989). High temperatures or prolonged heating alter sensory attributes in foods. Therefore, a proper time-temperature combination should be established to produce safe and organoleptically acceptable food products.

### **Metabolically injured organisms**

Pathogens may undergo metabolic injury when subjected to extrinsic factors like environmental stress. Environmental stress can be induced by sub lethal heat, freezing/freeze drying, irradiation, aerosolization, dyes, sodium azide, salts, heavy metals, antibiotics, essential

oils, and other chemicals, such as EDTA and sanitizing compounds (Jay et al., 2005). Metabolic injury results in the inability of a pathogen to form colonies on selective media that uninjured cells can tolerate and grow. Metabolic injured cultures can be repaired by placing them in a recovery medium (suitable nutrient broth) at the appropriate time and temperatures. The existence of the metabolically injured cells in foods and their recovery during culturing procedures is of great importance for both pathogenic organisms and spoilage organisms.

Nelson (1943) first demonstrated sub lethal stress effect on foodborne pathogens by an increased nutritional requirement of bacteria that had undergone heat treatment (metabolically injured organisms). These organisms may also manifest their injury via increased lag phases of the growth, increased sensitivity to a variety of selective media agents, damage to cell membranes and tricarboxylic acid (TCA) cycle enzymes, as well as ribosomal and DNA damage. In metabolically injured cells, damage to cell membrane, ribosomes, DNA and enzymes occurs. The lipid component of the cell membrane is the most likely target, especially in sub lethal thermal treatments (Hurst, 1977). Leakage of  $Mg^{+2}$  ions causes ribosomal damage (Hurst et al., 1978). Pyruvates and catalases are injury-repairing agents that help in growth revival of injured cells.

## **MULTIPLE HURDLE TECHNOLOGY APPROACH**

The growing demand for fresh, minimally processed foods by consumers has led to the need for natural food preservation methods such as the use of antimicrobial peptides to control the growth of foodborne pathogens that have no adverse effects on the consumer or the food itself. Incorporating “natural plant extracts” and reducing conventional chemical antimicrobials without compromising food safety and sensory properties would be a novel way in inhibiting these pathogens. Furthermore, these approaches would pave the control measures towards

becoming “green” as the consumers and processors are leaning towards “natural/green” food products.

Hurdle technology was first used in the mid 1980s by L. Leistner in Germany. Now it has become an increasingly popular microbial intervention technology to preserve food products. Foodborne, pathogen contamination can be reduced by following one or more approaches discussed in the control measures through multiple hurdle technology approach. The use of multiple environmental factors or techniques (i.e., pH, salt concentration, and temperature) and/or chemical agents like antimicrobial agents to control the microbial growth in foods is called multiple hurdle technology (Montville and Matthews., 2005; Jay et al., 2005).

Multiple hurdle technology targets the bacterial cell in different ways resulting in better control of the pathogen. Hurdle technology disturbs homeostatic process, which is fundamental to life. Hurdle technology “de-optimizes” several factors, setting one environmental parameter to the extreme limit for growth. For example, microbial inhibition can be obtained by limiting the water activity ( $a_w$ ) to  $< 0.85$  or limiting pH to 4.6. Hurdle technology can obtain similar inhibition at pH 5.2 and  $a_w$  of 0.92. These disturbances to the homeostasis demand the microbes to channel energy to maintain homeostasis. When the energy demands of homeostasis exceed the microbe’s energy producing capacity, the pathogen starved to death. Precise definitions and determination of the factors that permit and prevent growth of a given organism can be explained by advanced hurdle technology – growth/no, growth (G/NG) interface, where interaction between two or more parameters that completely inhibit the microbial growth was implicated (Mc Meekin et al., 2000).

### **Use of GSE and GTE in multiple hurdle approach**

Though plant extracts have demonstrated antimicrobial properties, they may not be sufficient alone to decontaminate and extend the shelf life of food products in case of pathogen contamination (Juneja et al., 2010). Multiple hurdle approach/technologies involving interventions like vacuum packaging, pH, storage temperatures, other antimicrobials like organic acid treatments such as lactates, acetates and diacetates, and post-process heat treatments along with potent natural plant extracts have food safety applications and contribute towards such increasing trends in the food industry (Juneja et al., 2010; Tsigarida et al., 2000).

### ***Synergistic action of grape seed and green tea extracts***

The antimicrobial activity of the plant extracts can be attributed to their action on the bacterial cell membrane (Cowan, 1999). Therefore, using the antimicrobial compounds that have similar action on the bacterial cell wall through a multiple hurdle approach could yield synergistic effects. Theivendran et al., (2006) have shown synergistic inhibitory effects of GSE (1 %) or GTE (1 %) in combination with nisin (10,000 IU/mL) compared to plant extracts alone against starving *L. monocytogenes* in PBS medium incubated at 37 °C. These synergistic activities may be due to facilitating the diffusion of major phenolic compounds in GTE (epicatechin, caffeic, benzoic, and syringic acid) or GSE (epicatechin, catechin, genistic, and syringic acid) through the pores formed in the pathogenic cell membrane caused by the activity of nisin (Theivendran et al., 2006). Furthermore, GSE (1 %), when combined with nisin (6,400 IU/mL), inhibited *L. monocytogenes* populations to undetectable levels (minimum detection limit was 100 CFU/g) in full fat turkey frankfurter formulations (21 % fat) when stored at both 4 °C and 10 °C (Sivaroban et al., 2007). Addition of grape seed and green tea extracts increased the shelf life of raw patties and other meat products stored under retail display conditions (Banon et al., 2007). Both the extracts (GTE and GSE) have an antibacterial effect in *in vitro* conditions

(Ahn et al., 2004). Incorporating major phenolic constituents in GTE or GSE along with nisin can demonstrate changes to the morphology and internal structures of *L. monocytogenes* cells using transmission electron microscopic studies (Sivaroban et al. 2008a) (Fig. 2.4 & 2.5). In our laboratory we have worked on various intervention strategies and multiple hurdle approaches using GTE and GSE, alone or combined with nisin, in various model systems that have potential food applications to enhance the food safety of the product (Table 2.2).

**Table 2.2**

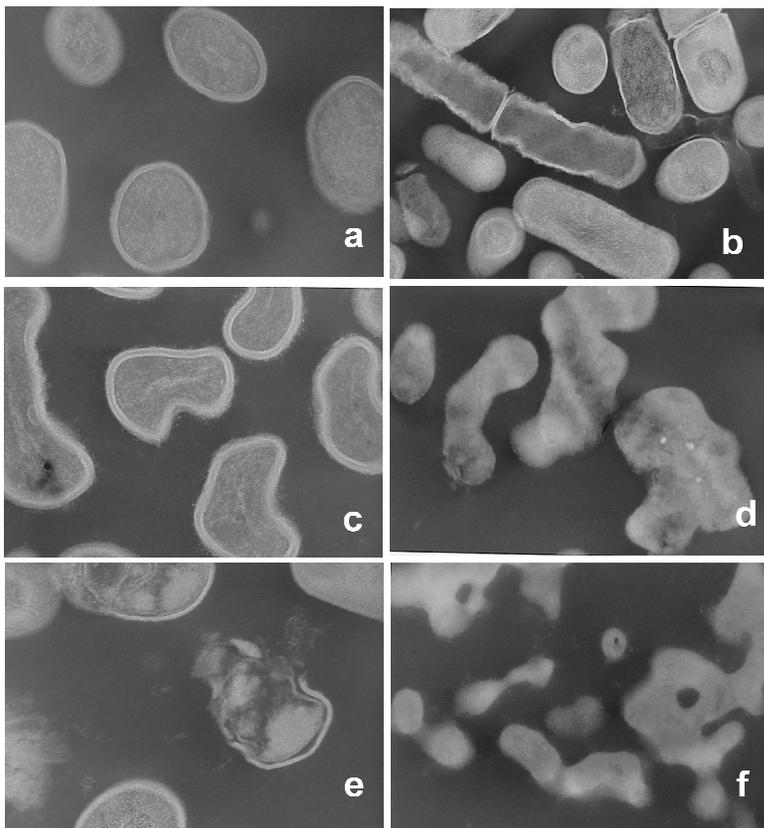
Summary of research findings involving green tea and grape seed extracts as antimicrobial components in multiple hurdle approach in various model systems.

Model system	Multiple hurdle approach	Significant findings	Reference
BHI broth culture	Tartaric acid (37.5 mM) + GTE (20 or 40 mg/mL) or GSE (20 or 40 mg/mL)	GTE alone (20 or 40 mg/mL) or in combination with tartaric acid (37.5mM) reduced <i>Salmonella</i> , <i>Listeria</i> , and <i>E. coli</i> by at least 3.5 log cfu/mL.	Over et al.,2009
Tryptic soy broth with 0.6 % yeast extract (TSBYE)	GSE (1 %) alone or in combination with nisin (6,400 IU) at 37 °C	No detectable levels of <i>L. monocytogenes</i> observed by 12 h of incubation. Minimum detection limit was 10 cfu/mL.	Sivarooban, et al., 2007
Turkey frankfurters	GSE (1 %) alone or in combination with nisin (6,400 IU) at 37 °C	No detectable levels of <i>L. monocytogenes</i> observed by 28 days of storage at 4 °C and 10 °C. Minimum detection limit was 100 cfu/g.	Sivarooban et al., 2008a
Soy protein isolate film	GSE (1 % w/w), nisin (10,000 IU/g), and EDTA (0.16 % w/w)	<i>Listeria monocytogenes</i> populations reduced by 2.9 log CFU/mL, while <i>E. coli</i> O157:H7 and <i>Salmonella</i> Typhimurium were reduced by 1.8 and 0.6 log cfu/mL, respectively.	Theivendran et al., 2006
Soy protein film coated turkey frankfurters	Nisin (10,000 IU) + GSE (1 %) or GTE (1 %)	Samples containing nisin combined with either GSE or GTE reduced <i>Listeria monocytogenes</i> population (7.1 cfu/g) by more than 2 log cfu/g after 28 days at 4 °C and 10 °C.	Gadang et al., 2008
Whey protein isolate coated turkey franks	GSE (0.5 %), nisin (6000 IU/g), EDTA (1.6 mg/mL), and Malic acid (1 %)	A combination of nisin, GSE, and malic acid reduced <i>L. monocytogenes</i> population (5.5 log cfu/g) to 2.3 log cfu/g and. <i>S. Typhimurium</i> population (6.0 log cfu/g) to approx. 1 log cfu/g in the samples with nisin, malic acid, GSE, and EDTA after 28 d at 4 °C.	

Spinach	Electrostatic spray of malic acid (2 %), GSE (2 %), and tartaric acid (2 %).	Malic acid and GSE provided 2.6 and 3.3 log cfu/g reductions of <i>Listeria and Salmonella</i> on the days 7 and 14 respectively.	Ganesh et al., 2010
Probiotics	Green tea extract (TEAVIGO™) and <i>Lactobacillus</i> spp. or <i>Bifidobacterium</i> in RCM (Reinforced Clostridial Broth).	Synergistic effect (reduction) of probiotics (4- 5 log cfu/ mL) and GTE (400 µg/mL) against <i>Staphylococcus aureus</i> (2 -15 fold) and <i>Streptococcus pyogenes</i> (3 – 30 fold).	Su et al., 2008
Edible <i>Gelidium corneum</i> -gelatin (GCG) films for packing pork loins	Green tea extract and packing	Pork chops packed with GCG film containing GTE (2.80 %) decreased <i>Escherichia coli</i> O157:H7 (0.69 to 1.11 log cfu/g) and <i>L. monocytogenes</i> populations (1.05 to 1.14 cfu/g) respectively when compared to control after 4 days of storage.	Hong et al., 2009.
Beef Patties	Sulphite (100 mg/kg) and GSE or GTE (300mg/kg)	Combination of low sulphite and GTE - 0.6 and 1.7 log cfu/g reductions in total viable counts and total coliform counts respectively on 9 <sup>th</sup> day of storage. Combination of low sulphite and GSE - 0.8 and 2.1 log cfu/g reductions in total viable counts and total coliform counts respectively on 9 <sup>th</sup> day of storage.	Banon et al., 2007

**Figure 2.4**

Transmission electron microscopy (TEM) of *L. monocytogenes* treated by nisin combined with either phenolics or grape seed extract (GSE) in TSBYE medium at 37 °C.



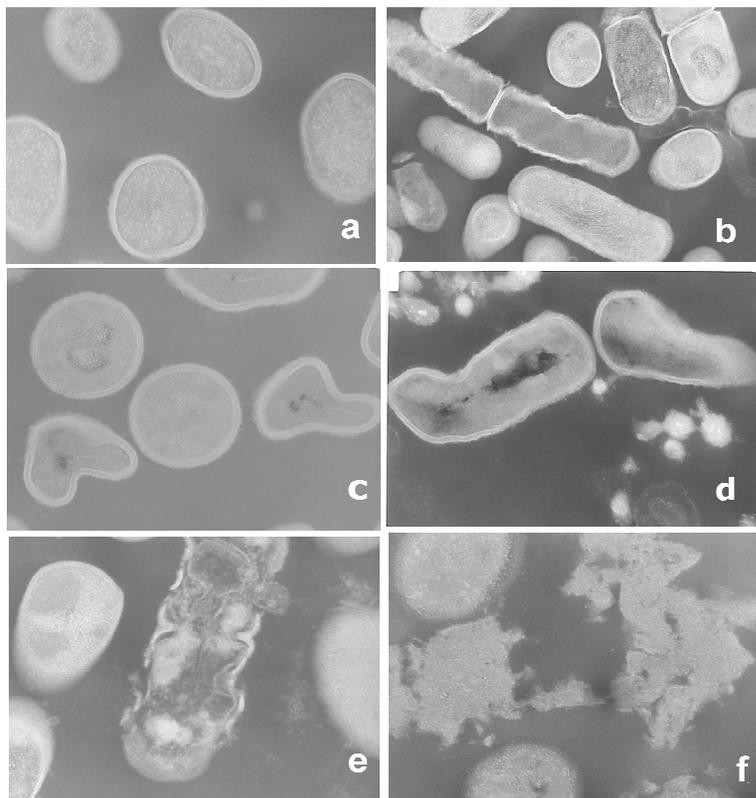
- a:** Control (without antimicrobials),
- b:** Nisin (6,400 IU/mL),
- c:** Pure phenolics (combination of epicatechin 0.02 % + catechin 0.02%),
- d:** GSE 1%,
- e:** Nisin (6,400 IU/mL) + pure phenolics (combination of epicatechin 0.02 % + catechin 0.02%),
- f:** Nisin (6,400 IU/mL) + GSE 1%

*L. monocytogenes* (approximately  $10^6$  cfu/mL) treated with and without antimicrobials in TSBYE; tryptic soy broth + yeast extract 0.6% Incubated for 3 h at 37 °C viewed under TEM (operating at 80 kV, x 50,000; Bar: 0.2  $\mu$ m).

Adapted from Sivaroban, T., Hettiarachchy, N.S. and Johnson, M.G. (2008a). Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extract. *J Food Prot*, 71, 2105-2109.

## Figure 2.5

Transmission electron microscopy (TEM) of *L. monocytogenes* treated by nisin combined with either phenolics or green tea extract (GTE) in TSBYE medium at 37 °C.



- a:** Control (without antimicrobials),
- b:** Nisin (6,400 IU/mL),
- c:** Pure phenolics (combination of epicatechin 0.02 % + caffeic acid 0.02%),
- d:** GTE 1%,
- e:** Nisin (6,400 IU/mL) + pure phenolics (combination of epicatechin 0.02 % + caffeic acid 0.02%),
- f:** Nisin (6,400 IU/mL) + GTE 1%

*L. monocytogenes* (approximately  $10^6$  cfu/mL) treated with and without antimicrobials in TSBYE; tryptic soy broth + yeast extract 0.6% Incubated for 3 h at 37 °C viewed under TEM (operating at 80 kV, x 50,000; Bar: 0.2  $\mu$ m).

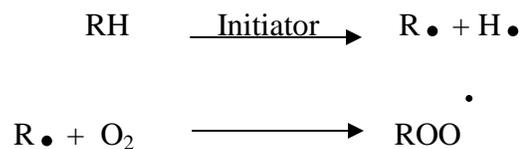
Adapted from Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. (2008a). Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extract. *J Food Prot*, 71, 2105-2109.

## LIPID OXIDATION

Fat is a major food constituent that influences the organoleptic characteristics of meat products. The basic functions of fats in foods are as sources of essential fatty acids, transport molecules of fat-soluble vitamins, and energy sources (Mela, 1990). The sensory properties of fat influence the texture, juiciness, and flavor of food products (Drewnowski, 1992). However, excess consumption of fat causes obesity, arteriosclerosis, and colon cancer (de Vries, 2007). Thus, low fat meat products have been a major consumer interest. Lipids, an important constituent in most foods, are susceptible to hydrolysis (lipolysis), oxidation, and other chemical processes that results in desirable and undesirable compounds and flavors (Alford et al., 1971). Fat decomposition and subsequent development of rancid flavors is due to hydrolysis and oxidation processes respectively.

Lipid oxidation is one of the major deteriorative chemical changes that would decrease the shelf life of a food product, and thus, decrease its overall acceptability (Cortinas et al., 2005). Oxidation of labile double bonds in polyunsaturated fatty acids (PUFA) produces secondary oxidative compounds such as hexanal, pentanal, heptanal, and octanal that are responsible for quality deterioration, warmed over flavors (WOF), and present health risks (Grun et al., 2006). The mechanism involved in lipid oxidation in foods can be discussed in three steps.

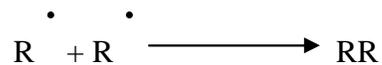
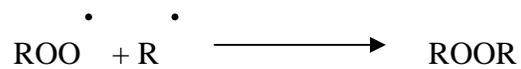
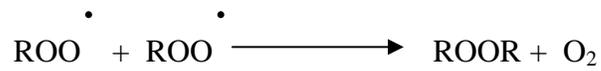
**Step 1: Initiation:** Loss of hydrogen radical (H.) from unsaturated fatty acid (RH) when reacted with oxygen (O<sub>2</sub>) in presence of light, heat, or trace metals to form peroxy radicals (ROO')



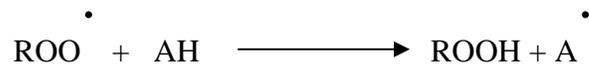
**Step 2: Propagation:** In this process, peroxy radicals react with more unsaturated fatty acids to form lipid hydro peroxides (ROOH).



**Step 3: Termination:** The oxidation chain process terminates when two peroxy radicals react to produce a non-radical species (ROOR or RR).



Antioxidant activity (AH) refers to the delay or inhibition of oxidation of lipids or other molecules by inhibiting the initiation or propagation step of the oxidative chain reactions or forming stable radicals (A<sup>•</sup>) which are either unreactive or form non-radical products (Huang et al., 2005).



Oxidation of meat products depends on PUFA composition of the fat of muscle and other tissues, fatty acids (mostly unsaturated) present in the muscle membrane, presence of pro-oxidants and/or transition metals such as iron in the form of heme pigment, lipid composition of feed, and finally the presence of antioxidants (Faustman and Cassens, 1990). Oxidative

deterioration of lipids in food produces hydroperoxides decompose to give [do the hydroperoxides decompose to give off these products such as aldehydes, ketones, acids, epoxides, and polymers. These compounds are responsible for unpleasant odors and tastes that reduce the overall acceptability of the meat by the consumer. The metmyoglobin levels involved in the oxidative processes and reductant enzymatic systems leads to the discoloration of the meat (Faustman and Cassens, 1990). Deteriorative effects on lipid oxidation are more pronounced in precooked meat, which causes “warmed-over flavors.”

Vitamin E found in the biological membranes exhibits antioxidant properties by protecting PUFA, which are extremely susceptible to oxidation. Deposition of vitamin E in chicken and turkey meat is different as turkey is less efficient in terms of deposition of  $\alpha$ -tocopherol both in fat and muscle (Marusich et al., 1994). Furthermore, the concentration of  $\alpha$ -tocopherol in turkey thigh meat is six times greater than its breast meat due to greater vascularity of the leg (Sheldon et al., 1984). Lower concentration of the  $\alpha$ -tocopherol in turkey than chicken meat renders turkey meat more susceptible to lipid oxidation, and thus explains the greater vitamin E requirements of this species as compared with chicken.

### ***Antioxidant properties – Green tea and grape seed extracts***

#### ***Green tea extract – Mechanism of action***

Polyphenolic compounds (mainly flavanoids) present in green tea extract have demonstrated potential antioxidant properties due to their redox potential that enable them to act in various forms such as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions in numerous food applications (Gramza et al., 2006). The active hydroxyl groups present in the molecular structure of polyphenols (Fig 2.1) are the active components of the green tea extract that can interact with the free radicals to inhibit lipid oxidation (Mitsumoto

et al., 2005). Furthermore, tea polyphenols can exhibit scavenging activity against free radicals (Rice-Evans et al., 1997), superoxide radicals, peroxyxynitrite, chelate copper, and iron, preventing metal catalysed free radical formation (Lin and Liang, 2000). Flavonoids present in plant extracts terminate the radical chain reactions that occur during the oxidation of triglycerides in food systems (fats, oils, and emulsions) and, thus, can act as free radical scavengers (Turkoglu et al., 2007). Presence of *O*-dihydroxy and *O*-hydroxyketo groups and C2-C3 double bonds are related to the scavenging activity of different catechin molecules (Rice-Evans et al., 1997). Thus, EGCG has the most scavenging activity of free radicals followed by ECG, EC, and EGC as the antioxidant functions of the tea catechins depend on the structure, position, and number of hydroxyl groups (Hu et al., 2001). In addition, concentration, solubility and the accessibility of the active groups to the oxidant, and the stability of the product play an important role in the antioxidant properties of the green tea extract (Guo et al., 1999).

#### ***Grape seed extract – Mechanism of action***

Recent literature has shown antioxidant properties of GSE both *in vivo* and *in vitro* (Yilmaz and Toledo, 2004). The antioxidant properties of GSE are primarily due to flavonoids that can perform scavenging action on free radicals (superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH)), metal chelating properties, reduction of hydroperoxide formation, and their effects on cell signaling pathways and gene expression (Sato et al., 1996; Soobrattee et al., 2005; Jacob et al., 2008). The presence of functional group - OH in the structure and its position on the ring of the flavanoid molecule (Fig 2.2) determines the antioxidant capacity (Arora et al., 1998). Addition of OH groups to the flavonoid nucleus will enhance the antioxidant activity, while substitution by -OCH<sub>3</sub> groups diminish the antioxidant activity (Fig 2.2) (Majo et al., 2008). Degree of polymerization of the procyanidins may also determine the antioxidant activity

–the higher the degree of polymerization, the higher the antioxidant activity (Spranger et al., 2008). Among different parts of grape plant, grape seeds exhibit the highest antioxidant activity followed by the skin and the flesh (Pastrana-Bonilla et al., 2003). The antioxidant potential of GSE is twenty and fifty fold greater than vitamin E and C, respectively (Shi et al., 2003).

## **PHYSICOCHEMICAL PROPERTIES**

Addition of phenolic-rich plant extracts such as GTE and GSE (light brown and red color respectively) can interact with minerals and proteins and, thus, may affect physicochemical (pH, water activity, color, and texture). Organic acids such as acetates, lactates, and diacetates are being used as antimicrobial ingredients in various foods, including RTE meats, particularly because of their ability to reduce *L. monocytogenes* in ready-to-eat meat products (Alvarado and McKee, 2007). Mechanism of action of these organic acids is usually by intracellular acidification i.e. lowering the pH (Hunter and Segal, 1973) and lowering  $a_w$  in the system (Miller and Acuff, 1994). Reduction of pH and  $a_w$  in processed meat formulations such as hotdogs have a significant effect on color and texture of the final product in addition to extending shelf life. The appearance of cooked RTE meats can be influenced by pH, meat source, fat content, and added ingredients. These factors change the ratio of different forms of myoglobin, the main pigments responsible for the ultimate color of meat (King and White, 2006).

Color and appearance of food products are major factors in consumer-purchase decisions because they are believed to be indicators of meat quality (Brewer et al., 2002). Meat color is due to the pigment myoglobin that binds to oxygen in the muscle until it is needed for metabolic processes. Myoglobin is composed of a protein (globin) and an iron-containing group (heme) (Lawrie, 2002). The iron can exist in the reduced ( $Fe^{2+}$ ; deficient in two electrons) or oxidized ( $Fe^{3+}$ ; deficient in three electrons) states. In food, iron can be a prooxidant. It can catalyze the

breakdown of lipid oxidation products by promoting free radical formation from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Brewer et al., 2002). Phenolic-rich plant extracts such as GTE and GSE have strong H• donating activity that can effectively scavenge peroxides and reactive oxygen species (Lugasi et al., 1995). The free-radical-scavenging potential of natural polyphenolic compounds depends on the number and arrangement of free –OH groups on the flavonoid skeleton (Kondo et al., 2001).

## **SENSORY PROPERTIES**

Phenolics contribute to the bitterness and astringency because of the interaction between phenolics, mainly procyanidins, and the glycoprotein in saliva and thus may influence sensory or organoleptic properties (appearance, juiciness, flavor, texture, and overall impression) (Dai and Mumper, 2010). Factors driving the consumer acceptance of any food product are color, moisture retention (juiciness), tenderness, and flavor (Jeremiah, 1978). This concept was further extended by subdividing into appearance and eating quality or palatability factors (Asghar and Pearson, 1980). Visual appearance is influenced by the color and final presentation of the product, which ultimately influences the purchasing decision of the consumer. On the other hand, consumer satisfaction is perceived by eating quality. There is general agreement in the literature that eating satisfaction is based on the interaction amongst tenderness, juiciness, and flavor, though juiciness and flavor are not as influential as tenderness (Koohmarie, 1996).

Juiciness depends on the amount of water retained in a cooked meat product. It helps in softening meat, making it easier to chew, and stimulates saliva production in the mouth. Water retention and lipid content can impact tenderness and also determines juiciness (Blumer, 1963). The perception of flavor and aroma are intertwined and rely on the smell through the nose and on the sensations of salty, sweet, sour, and bitter on the tongue. Flavor is the result of the

combination of compounds in the tissue and those produced by the Maillard reaction during cooking (Blumer, 1963). Flavor is one of the important attributes in food quality and sensory evaluation attributed to factors such as components of the muscle (water soluble, sulfur, and nitrogen components), type of meat, intramuscular fat content, diet regimen, cooking methods, aging, and refrigeration of the meat (Dawson et al, 1987). Texture is the most important quality attribute (Olsson et al., 1994) influencing the consumer acceptability as rated by the consumer (Deatherage, 1963; Lawrie, 1998). Texture can be attributed to a perception of meat, such as softness to tongue and cheek, resistance to tooth pressure, fragmentation of the food particles, adhesion, and residual after chewing (Breene, 1978).

*Acceptance* refers to the degree of liking or disliking for a particular product (Stone et al 1993). This is inferred from a person's ratings on scales or some other behavioral measure. It can be evaluated for a single product. *Preference* refers to a choice made by panelists among several products on the basis of liking or disliking (Stone et al., 1993). This is inferred from a person's choice among a set of alternatives (two or more products). When only two products are used, it is a simple paired-test. When more than two are used, it is a ranking test. "Hedonics" or "hedonic testing" is the common term applied to both acceptance and preference testing (Young 1961).

### ***Sensory evaluation***

Sensory evaluation is a scientific discipline used to evoke measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (Anonymous, 1975). In earlier days, sensory evaluation was referred as organoleptic testing and its development had emerged due to the competition and the evolution of processed and formulated foods (Pfenninger, 1979). This allowed the processors to obtain detailed knowledge about their contemporary products. Among several sectors of the consumer products industries, the food and beverage sectors provided much early support for

sensory evaluation. Historically, sensory evaluation received additional impetus through the US Army Quartermaster Food and Container Institute, which supported research in food acceptance for the armed forces (Peryam et al., 1954). Sensory information is used as a driving force for marketing decisions as it allows processors to identify and quantitatively model the key drivers for product's acceptance. In the early stages of the growth of the food-processing industry, numerical values were assigned to the scorecards (Hinreiner, 1956). For example the 100-point butter scorecard, the ten-point oil scale, and the twenty-point wine scorecard had specific numbers that implied levels of product acceptance. While scoring procedures were used as early as the 1940s (Baten, 1946), primary emphasis was given to the use of various paired procedures for assessing product differences and preferences. Rank-order procedures and hedonic scales became more common in the mid to late 1950s (Stone and Sidel, 1993).

### ***Role of sensory evaluation***

The main objective is to provide valid and reliable information to production, research and development, and marketing and quality control to make profitable decisions about the perceived sensory properties of the food products (Meilgaard, 1991). The ultimate goal of any consumer test should be to find the most cost-effective and efficient method to obtain the valuable consumer insights about the food products. Further, cost savings can be realized by correlating as many sensory properties as possible with instrumental, physical, or chemical analysis.

### **Consumer test**

A variety of sensory tools can be used in product evaluation to assess perceived intensity and acceptability of food products. Consumer tests provide information regarding measurement of tenderness and affective responses (Schilling et al., 2003). Two common and reliable ways to

measure sensory evaluation of food products widely used in the industry are consumer testing and descriptive analysis (Szczesniak, 1987; Stone and Sidel, 1993; Meilgaard et al., 1999). The 9-point hedonic scale is probably the most useful method for consumer's liking and preference and runs from "dislike extremely" to "likely extremely" with a mid-neutral category "neither like or dislike" at the center of the scale, which requires only a minimum verbal ability for reliable results (ASTM, 1968; Mahoney, 1986). Just-About-Right (JAR) scales are commonly used to assess consumer expectations of a product attribute to provide directional information to product developers (Robinson et al., 2005).

### ***Hedonic scale***

The hedonic scale is an outcome of several efforts to assess the acceptability of military foods and was described and developed by Jones et al. (1955) and by Peryam and Pilgrim (1957). It has significant importance in general applicability to the measurement of several hundred-food products' acceptance–preference (Peryam et al., 1960). The 9-point hedonic scale runs from "dislike extremely" to "likely extremely" with a mid neutral category "neither like or dislike" at the center of the scale. This test relies on people's ability to communicate their feelings of like or dislike. Hedonic testing is popular because it may be used with untrained people as well as with experienced panel members. A minimum amount of verbal ability is necessary for reliable results (O Mahony, 1986).

In hedonic testing, samples are presented in succession and the subject is told to decide how much he likes or dislikes the product and to mark the scales accordingly. The nature of this test is its relative simplicity. The instructions to the panelist are restricted to procedures, and no attempt is made at direct response. The subject is allowed, however, to make his own inferences about the meaning of the scale categories and determine for himself how he will apply them to

the samples (Jones, 1955; Peryam and Pilgrim, 1957). A separate scale is provided for each sample in a test session. The scales may be grouped together on a page, or be on separate pages (ASTM, 1968).

The hedonic scale is anchored verbally with nine different categories ranging from “like extremely” to “dislike extremely.” These phrases are placed on a line-graphic scale either horizontally or vertically. Many different forms of the scale may be used with success; however, variations in the scale form is likely to cause marked changes in the distribution of responses and ultimately in statistical parameters such as means and variances (ASTM, 1968). Hedonic ratings are converted to scores and treated by rank analysis or analysis of variance. As mentioned earlier, hedonic scales are used with both experts and untrained consumers, with the best results obtained with an untrained panel (Amerine et al., 1965). The ratings’ labels obtained on a hedonic scale may be affected by many factors other than the quality of the test samples. Characteristics of the subjects, the test situation, attitudes, or expectations of the subjects can all have a profound effect on results. A researcher needs to be cautious about making inferences on the bases of comparison of average ratings obtained in different experiments (ATSM, 1968).

A variety of sensory tools can be used in product evaluation to assess perceived intensity and acceptability of food products. Many other tests, besides hedonic scales, are used in the sensory evaluation of a food product. Determining the type of research that is being performed, and the type of evaluation that is needed is crucial in obtaining accurate results from a sensory project.

### ***Just-About-Right Scale***

Just-About-Right (JAR) scales are commonly used to assess consumer expectations of a product attribute to provide directional information to product developers (Robinson et al., 1999). While

JAR scales are most frequently used in larger scale consumer testing, they are an ineffective substitute for well-designed experiments or good sensory descriptive data (Stone et al 1983). These scales combine attribute intensity and preference in a single response, and are highly susceptible to interpretive and/or semantic errors because the product attribute to be measured is given a name. These bipolar scales, having three or five categories, are usually anchored with statements of too much, too little, or about right for each attribute.

## **POTENTIAL APPLICATIONS OF GREEN TEA AND GRAPE SEED EXTRACTS IN FOOD PRODUCTS**

Green tea extract has been used in various food applications such as bread (Wang and Zhou, 2004), extra virgin olive oil (Rosenblat et al., 2008), meat, sausages and fish (Bozkurt et al., 2006; Martinez et al., 2006; Alghazeer et al., 2008), dehydrated apple products (Lavelli et al., 2010), rice starch products to prevent retrogradation of starch (Wu et al., 2009), and biscuits (Mildner-Szkodlarz et al., 2009).

Grape seed extracts have demonstrated antioxidant and antimicrobial activities alone or in combination with other hurdle technologies in various food applications such as tomatoes, frankfurters, raw and cooked meat and poultry products, and fish (Bisha et al., 2010; Gadang et al., 2008; Ahn et al., 2002, Banon et al., 2007, Brannan and Mah, 2007; Brannan, 2009; Carpenter et al., 2007; Lau et al., 2003; Mielnik et al., 2006; Nissen et al., 2004; Pazos et al., 2004).

### **Antimicrobial properties**

Antimicrobial effect of plant extracts depends on pH and solubility of the extract in the model systems (Hao et al., 1998). Plant extracts that have low pH are more effective in inhibiting the microbial growth (Conner and Beuchat, 1984). In certain foods, the antimicrobial effect of

plant extracts increase with increasing solubility (Shelef et al., 1984; Ismaiel and Pierson, 1990, Mbat et al., 2006).

### ***Green tea extract***

Green tea extract (GTE) demonstrated inhibitory properties against major foodborne pathogens including *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter jejuni*, and others including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella* Enteritidis, *Shigella flexneri*, *Shigella dysenteriae*, and *Vibrio cholera* (Toda et al., 1991; An et al., 2004; Gadang et al., 2008; Hamilton and Miller, 1995). Furthermore, the antimicrobial activity of tea remains functional even at high temperatures (100 °C / 60 min) or at pasteurization temperatures (121 °C/ 15 min) (Diker and Hascelik, 1994; Oh et al., 1999). Polyphenols present in GTE have shown inhibitory effects on bacteria causing dental caries *Streptococcus mutans* at a minimum inhibitory concentration of 250µg/mL (Sakanka et al., 1989). Irradiation of green tea polyphenols could enhance the antimicrobial properties against *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, and *Streptococcus mutans* (An et al., 2004). Chinese green tea extracts (*Camellia sinensis* L.) at 500 µg/mL showed strong inhibitory effect (44 to 100 %) on major foodborne pathogens (*Listeria*, *Salmonella*, *Staphylococcus*, and *Bacillus species*) (Si et al., 2006). Over et al. (2009) demonstrated that GTE alone (20 or 40 mg/mL) or in combination with tartaric acid (37.5mM) reduced *Salmonella*, *Listeria*, and *E. coli* by at least 3.5 log cfu/mL in broth culture studies. The antimicrobial activities of GTE when combined with bacteriocins like nisin have demonstrated more effectiveness than alone against major foodborne pathogen like *Listeria monocytogenes*. This may be due to the synergistic mechanism of action of nisin and catechins present in green tea extract (Sivarooban et al., 2008b).

### ***Grape seed extract (GSE)***

Antimicrobial properties of GSE have been evaluated against *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Bacillus cereus*, *Enterobacter sakazakii*, *Escherichia coli* O157:H7, *Aeromonas hydrophila*, and other foodborne pathogens, both *in vitro* and to a limited extent in foods (Ahn et al., 2004, Anastasiadi et al., 2009; Kim et al., 2009; Rhodes et al., 2006; Sivarooban et al., 2007). Phenolic compounds extracted from defatted grape seed extract have demonstrated inhibitory effects on *Staphylococcus aureus* and *Escherichia coli* (Rotava et al., 2009). GSE showed inhibition of *Staphylococcus aureus* after forty-eight hours and *Aeromonas hydrophila* after one hour (Bayder et al., 2006). Minimum inhibitory concentration of GSE for antilisterial activity was determined as 0.26 mg GAE/L (Anastasiadi et al., 2009).

Jayaprakasha et al. (2003) investigated the antimicrobial properties of GSE against Gram-positive (*Bacillus* and *Staphylococcus*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) and found that GSE inhibited bacterial growth at 340–390 mg GAE/L Gram (+) and 475–575 mg GAE/L Gram (–). Grape seed extract (1% w/w) also demonstrated antimicrobial activities against Gram-negative bacteria such as *E. coli* O 157:H7 and *S. Typhimurium* in cooked ground beef treatments (Ahn et al., 2007). The principal antibacterial compound found in methanol extract from grape seeds is gallic acid that had shown inhibitory effects on *E. coli* and *S. Enteritidis* (Shoko et al., 1999). GSE can also be used in preventing periodontal diseases by exhibiting bacteriostatic effects on the oral anaerobes (*F. nucleatum* and *P. gingivalis*) at a concentration of 2000 µg/ml (Furiga et al., 2009).

### **Antioxidant properties**

#### ***Green tea extract (GTE)***

Green tea catechins are natural compounds that have GRAS (Generally Recognized As Safe) status and have demonstrated antioxidant properties in various food applications. Incorporating green tea extract as a food additive, due to antioxidant activities, is a growing interest in the food industry. Green tea extract can improve the marketing potential of various food products such as cereals, cakes, dairy products, instant noodles, confectionary, ice cream, and fried snacks (Yang et al., 1995; Jiang et al., 1995; Wang et al., 2000). The anti-cariogenic and the deodorizing effect of the catechins can provide natural benefits to oral health products like toothpastes, mouthwashes, chewing gums, and breath fresheners (Lee et al., 2004; Miki et al., 1992).

Tea catechins have demonstrated higher antioxidant properties against lipid oxidation of cooked patties when compared to ginseng, mustard, rosemary, sage, butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), and vitamins C and E (Sullivan et al., 2004). However, the inhibition of lipid oxidation in meats including red meat, poultry, and fish was proven to be dose dependent. Addition of tea catechins at levels of 200 - 400 mg/kg have demonstrated the inhibitory effects on lipid oxidation significantly in red meat and poultry patties (Tang et al., 2001; Mitsumoto et al., 2005). This could be explained by the ability of tea catechins to bind the iron component of myoglobin that would help in delaying lipid oxidation by reacting with free radicals (Mitsumoto et al., 2005). At these levels, there were no significant effects on the sensory attributes such as flavor, taste, tenderness, and overall acceptability. Nevertheless, a negative impact on the color of raw and cooked chicken, and beef patties could influence the consumer appeal of the products. Supplementation of poultry diets with tea catechins (300 mg/kg feed) delayed lipid oxidation in chicken meat compared to controls and dietary vitamin E (200 mg/kg feed) (Tang et al., 2000).

Tea catechins demonstrated synergistic action on reducing lipid oxidation when combined with rosemary and sage at levels less than 0.5% in raw and cooked pork patties produced from frozen pork meat, and thus can be used as natural antioxidants (McCarthy et al., 2001a, b). High doses (1000 mg/kg) of green tea ethanolic extract can delay the loss of redness in raw pork patties throughout storage (Jo et al., 2003). Muscle foods rich in unsaturated fatty acids (e.g. arachidonic acid), such as fish, are more susceptible to lipid oxidation than poultry and red meats for which a high concentration of tea catechins (> 300 mg/kg) is required to inhibit lipid oxidation. Flavanoid compounds have stronger antioxidant and free radical scavenging activities than vitamins C and E (Wiseman et al., 1997; Vinson et al., 1995). Tea extracts also have the ability to inhibit the human salivary amylase; therefore, consuming tea could reduce cariogenic potential of starch rich foods such as crackers and cakes (Zhang and Kashlet, 1998).

#### ***Grape seed extract (GSE)***

Grape seed extracts have shown antioxidant activities both *in vivo* and *in vitro*, and in various meats (Brannan and Mah, 2007; Cos et al., 2004; Hu et al., 2004; Shaker, 2006). The suggested antioxidant activity *in vivo* include stimulating enzyme production of nitric oxide, oxygen radical scavenging, and inhibition of nitrositive stress (Bagchi et al., 2000., Roychowdury et al., 2001a, b). In meat systems, GSE demonstrates the antioxidant activity by reducing the amount of primary lipid oxidation products (e.g. lipid hydro peroxides and hexanal) and secondary lipid oxidation products (e.g. thiobarbituric acid reactive substances – TBARS) (Brannan and Mah, 2007). GSE has reduced rancid flavor development and antioxidant activities in various meat products like raw beef, cooked beef, raw and cooked pork patties, turkey, fish oil and frozen fish, and ground chicken breast and thigh meat (Ahn et al., 2002, Banon et al., 2007;

Brannan and Mah, 2007; Brannan, 2009; Carpenter et al., 2007; Lau et al., 2003; Mielnik et al., 2006; Nissen et al., 2004; Pazos et al., 2004).

The minimum concentration level of GSE required to produce an antioxidant effect in cooked pork was 400  $\mu\text{g/g}$  and 0.1% (w/w) in ground chicken to reduce the TBARS (Lau and King, 2003). The antioxidant activity of GSE is concentration dependent between 0.02% and 0.1% (Ahn et al., 2002). Grape seed extract at 0.1% (w/w) is an effective radical scavenger in muscle tissues and shown to reduce the secondary oxidation products (TBARS and head space hexanal) in beef, chicken, and turkey during refrigerated storage (Ahn et al., 2007; Mielnik et al., 2006; Rababah et al., 2006). At this level (0.1% w/w), GSE can be used as an effective antioxidant in both raw and cooked meat systems. Addition of GSE ( $\geq 1000 \mu\text{g/g}$ ) results in a minor increase ('a' – redness values) in the surface color of raw meat and retention (due to anthocyanins present in GSE) in cooked meat, which may have a negative impact on consumer preference based on color of the meat product; although, this does not affect the eating quality (Ahn et al., 2007; Carpenter et al., 2007; Rojas and Brewer, 2007). Addition of GSE (6000 ppm) does not change flavor scores in irradiated and non-irradiated whole chicken breasts (Rababah et al., 2005). Furthermore, there was no effect of GSE (0.1% w/w) on pH, yield, and water activity in ground chicken breast samples (Brannan, 2009).

The overall goal of this study is to use natural plant extracts (green tea and grape seed extracts) and to reduce the chemical antimicrobials such as lactates and diacetates used in RTE high and low fat chicken and turkey hotdogs to minimum level and to inhibit the growth of *Listeria monocytogenes*. The objectives are:

1. Investigate the effect of conventional chemical preservatives used in hotdog on reducing or eliminating *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  cfu/g levels in ready-to-eat high and low fat chicken and turkey hotdogs under normal and vacuum packaging during storage at 4 °C.
2. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA on inhibiting *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  cfu/g levels in ready-to-eat high and low fat chicken and turkey hotdogs under vacuum packaging during storage at 4 °C.
3. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA and heat treatment on inhibiting *Listeria monocytogenes* (V7 serotype 1/2a), inoculated at  $10^4$  cfu/g levels in ready-to-eat high and low fat chicken and turkey hotdogs under vacuum packaging during storage at 4 °C.
4. Investigate the effect of reducing the conventional chemical preservatives in hotdog and incorporating nisin/green tea extract/grape seed extract/combinations and EDTA on physicochemical and sensory properties of ready-to-eat high and low fat chicken and turkey hotdogs during storage at 4 °C.

## REFERENCES

- Abee, T., Rombouts, F.M., Hugenholtz, J., Guihard, G. and Letellier, L., 1994. Mode of action of nisin Z against *Listeria monocytogenes* Scott A grown at high and low-temperatures. *Appl Environ Microbiol*, 60, 1962–1968.
- Abee, T. 1995. Pore-forming bacteriocins of gram-positive bacteria and self protection mechanisms of producer organisms. *FEMS Microbiol Lett*, 129, 1 -10.
- Adamczak, L., Rucinski, M. and Slowinski, M. 1999. Niskotluszczowe produkty miesne. *Mieso i Wedliny*, 1, 26-29 (in Polish).
- Ahmed, N. M., Conner, D. E. and Huffman, D. L. 1995. Heat resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. *J Food Sci*, 60, 606-610.
- Ahn, Y.J., Kawamura, T., Kim, M., Yamamoto, T. and Mitsuoka, T. 1991. Tea polyphenols: selective growth inhibitors of *Clostridium* spp. *Agric Biol Chem*, 55, 1425-1426.
- Ahn, J., Grun, I.U. and Fernando, L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J Food Sci*, 67, 1364–1369.
- Ahn, J., Grun, I.U. and Mustapha, A. 2004. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J Food Prot*, 67, 48–55.
- Ahn, J., Grun, I.U. and Mustapha, A. 2007. Effects of plant extracts on microbial growth, colour change, and lipid oxidation in cooked beef. *Food Microbiol*, 24, 7–14.
- Alford, J.A. and Elliot, L.E. 1960. Lipolytic activity of micro-organisms at low and intermediate temperatures. I. Action of *Pseudomonas fluorescens* on lard. *Food Res*, 25, 296.
- Alford, J.A. and Pierce, D. A. 1963. Production of lipase by *Pseudomonas fragi* in a synthetic medium. *J Bact*, 86, 24-29.
- Alford, J.A., Smith, J. L. and Lilly, H.D. 1971. Relationship of microbial activity to changes in lipids of foods. *J Appl Bact*, 34, 133-146.
- Alghazeer, R., Saeed, S. and Howell, N.K. 2008. Aldehyde formation in frozen mackerel (*Scomber scombrus*) in the presence and absence of instant green tea. *Food Chem*, 108, 801–810.
- Amidor, T. 2009. Wholesome green tea. *Food Product Design*, July 27.
- An, B. J., Kwak, J. H., Son, J. H., Park, J. M., Lee, J. Y., Jo, C., et al. 2004. Biological and antimicrobial activity of irradiated green tea polyphenols. *Food Chem*, 88, 549 –555.

- Anastasiadi, M., Chorianopoulos, N.G., Nychas, G.J.E. and Haroutounian, S.A. 2009. Antilisterial activities of polyphenol-rich extracts of grapes and vinification by-products. *J Agri Food Chem*, 57, 457–463.
- Angelidis, A.S. and Smith, G.M. 2003. Role of the glycine betaine and carnitine transporters in adaptation of *Listeria monocytogenes* to chill stress in defined medium. *Appl Environ Microbiol*, 69, 7492–7498.
- Arora, A., Nair, M.G. and Strasburg, G.M. 1998. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radical Biol Med*, 24, 1355–1363.
- Asghar, A. and Pearson, A.M. 1980. Influence of ante- and postmortem treatments upon muscle composition and meat quality. In *Advances in Food Research*, 26, pp. 53-213, Academic Press, New York.
- Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M. and Booren, A.M. 1988. Perspectives on warmed over flavor. *Food Technol*, 42, 6, 102–108.
- ASTM. 1968. Manual on sensory testing methods, No. 434. Philadelphia, Pa: American Society for Testing and Materials.
- Auger, C., Al-Awwadi, N., Bornet, A., Rouanet, J. M., Gasc, F., Cros, G. and Pierre-Louis, T. 2004. Catechins and procyanidins in Mediterranean diets. *Food Res Int*, 37, 233–245.
- Bagchi, D., Bagchi, M., Stohs, S. J., Das, D. K., Ray, S. D., Kuszynski, C. A., Joshi, S.S., Pruess, H.G. 2000. Free radicals and grape seed proanthocyanidin extract: Importance in human health and disease prevention. *Toxicology*, 148, 187–197.
- Balentine, D.A., Wiseman, S.A. and Bouwens, L.C.M. 1997. The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr*, 37, 693 -704.
- Banon, S., Diaz, P., Rodriguez, M., Garrido, M.D. and Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Sci*, 77, 626–633.
- Barmpalia, I.M., Geornaras, I., Belk, K.E., Scanga, J.A., Kendall, P.A., Smith, G.C. and Sofos, J. N. 2005. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10 °C. *Food Microbiol*, 22, 205-211.
- Baten, W. D. 1946. Organoleptic tests pertaining to apples and pears. *J Food Sci*, 11, 84-94.
- Baydar, N.G., Sagdic, O., Ozkan, G. and Cetin, S. 2006. Determination of antibacterial effects and total phenolic contents of grape (*Vitis vinifera*) seed extracts. *Int J Food Sci*, 41, 799–804.
- Bayles, D.O., Annous, B.A., Wilkinson, B.J. 1996. Cold stress proteins induced in *Listeria monocytogenes* in response to temperature down shock and growth at low temperatures. *Appl. Environ Microbiol*, 62, 1116–1119.

- Beales, N., 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: a review. *Comp Rev Food Sci Food Safety*, 3, 1–20.
- Beauchemin, M. 1990. Sous-vide technology. *Proc Recipr Meat Conf*, 43, 103-107.
- Benech, R.O., Kheadr, E.E., Laridi, R., Lacroix, C. and Fliss, I. 2002. Inhibition of *Listeria innocua* in cheddar cheese by addition of nisin Z in liposomes or by in situ production in mixed culture. *Appl Environ Microbiol*, 68, 3683–3690.
- Bentivegna, S.S. and Whitney, K.M. 2002. Subchronic 3-month oral toxicity study of grape seed and grape skin extracts. *Food and Chem Toxicol*, 40, 1731–1743
- Berry, B.W. 1992. Low fat level effects on sensory, shear, cooking, and chemical properties of ground beef patties. *J Food Sci*, 57, 537-540.
- Beuchat, L.R., Brackette, R.E. and Doyle, M.P. 1994. Lethality of carrot juice to *Listeria monocytogenes* as affected by pH, sodium chloride, and temperature. *J Food Prot*, 57, 470–474.
- Bisha, B., Weinsetel, N., Brehm-Stecher, B.F. and Mendonca, A. 2010. Antilisterial effects of gravinol-s grape seed extract at low levels in aqueous media and its potential application as a produce wash. *J Food Prot*, 73, 266-273.
- Blumer, T.N. 1963. Relationship of marbling to the palatability of beef. *J Anim Sci*, 22, 771.
- Boziaris., I.S. and Adams, M.R. 1999. Effect of chelators and nisin in situ on inhibition and inactivation of gram negatives. *Int J Food Microbiol*, 53, 105-113.
- Bradford, D.D., Huffman, D.L., Egbert, W.R. and Jones, W.R. 1993. Low fat fresh pork sausage patty stability in refrigerated storage with potassium lactate. *J Food Sci*, 58, 488–491.
- Brannan, R.G. and Mah, E. 2007. Grape seed extract inhibits lipid oxidation in muscle from different species during refrigerated and frozen storage and oxidation catalyzed by peroxy nitrite and iron/ascorbate in a pyrogallol red model system. *Meat Sci*, 77, 540–546
- Brannan, R.G. 2008. Effect of grape seed extract on physicochemical properties of ground, salted, chicken thigh meat during refrigerated storage at different relative humidity levels. *J Food Sci*, 73, 36–40.
- Brannan, R.G. 2009. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. *Meat Sci*, 81, 589–595.
- Branen, J.K. and Davidson, P.M. 2004. Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin. *Int J Food Microbiol* 90, 63–74.

- Brewer, M.S., Rostogi, B.K., Argoudelis, L. and Sprouls, G.R. 1995. Sodium lactate/sodium chloride effects on aerobic plate counts and color of aerobically packaged ground pork. *J Food Sci*, 60, 58–62.
- Brewer, M.S. 2004. Irradiation effects on meat color: A review. *Meat Sci*, 68, 1-17.
- Broughton, J. D. 2005. Nisin as a food preservative. *Food Australia*, 57, 525-527.
- Buchanan, R.L. and Doyle, M.P. 1997. Foodborne Disease Significance of *Escherichia coli* O157:H7 and other Enterohemorrhagic *E. coli*. *IFT Scientific status summary*, 51, 1-8
- Buchanan, R.L. and Whiting, R.C. 1998. Risk assessment: a means for linking HACCP plans and public health. *J Food Prot*, 61, 1531–1534.
- Buchanan, R., Damert, W., Whiting, R. and M. Van Schothorst. 1997. Use of epidemiological and food survey data to estimate a purposefully conservative dose-response relationship for *Listeria monocytogenes* levels and incidence of listeriosis. *J Food Prot*, 60, 918-922.
- Buchanan, R.L. and Bagi, L.K. 1997. Effect of water activity and humectant identity on the growth kinetics of *Escherichia coli* O157:H7. *Food Microbiol*, 10, 303-315
- Buchman, G.W., Banergee, S. and Hansen, J.N. 1988. Structure, expression and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. *J Biol Chem*, 263, 1620 – 1626.
- Burdock, G.A. 2005. Fenaroli's Handbook of Flavor Ingredients. Fifth edition, CRC Press, Boca Raton, FL, USA
- Calderon-Miranda, M.L., Barbosa-Canovas, G.V. and Swanson, B.G., 1999a. Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *Int J Food Microbiol*, 51, 7–17
- Calderon-Miranda, M.L., Barbosa-Canovas, G.V. and Swanson, B.G., 1999b. Inactivation of *Listeria innocua* in skim milk by pulsed electric fields and nisin. *Int J Food Microbiol*, 51, 19–30
- Cannarsi, M., Baiano, A., Sinigaglia, M., Ferrara, L., Baculo, Rodolfo. 2008. Use of nisin, lysozyme and EDTA for inhibiting microbial growth in chilled buffalo meat. *Int J Food Sci Technol*, 43, 573-578.
- Carpenter, R., O'Grady, M. N., O'Callaghan, Y. C., O'Brien, N. M. and Kerry, J. P. 2007. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Sci*, 76, 604–610
- CDC (Centers for Disease Control and Prevention). 1999. Multistate outbreak of listeriosis-United States, *Morbidity and Mortality Weekly Report*, 47, 1117-1118.

- CDC (Centers for Disease Control and Prevention). 2000. Multistate outbreak of listeriosis-United States. *Morbidity and Mortality Weekly Report*, 47, 1129-1130.
- CDC (Centers for Disease Control and Prevention). 2002. Public Health Dispatch: Outbreak of listeriosis-Northeastern United States, *Morbidity and Mortality Weekly Report*, 51, 950-951
- Chen, Y., Ross, W.H., Scott, V.N. and Gombas, D.E. 2004. *Listeria monocytogenes*: low levels equal low risk. *J Food Prot*, 66, 570-577
- Cheng, Q.K. and Chen, Z.M. 1994. 'Tea and Health', Press of Chinese Agricultural Sciences, Beijing, China
- Chirife, J. and Fontan, C.F. 1980. Prediction of water activity of aqueous solutions in connection with intermediate moisture foods: experimental investigations of the aw lowering behavior of sodium lactate and some related compounds. *J Food Sci*, 45, 802-804.
- Choi, S.H. and Chin, K.B. 2003. Evaluation of sodium lactate as a replacement for conventional chemical preservatives in comminuted sausages inoculated with *Listeria monocytogenes*. *Meat Sci*, 65, 531-537
- Christine, D. Wu and Guo-Xian, Wei. 2002. Tea as a Functional Food for Oral Health. *Nutrition Burbank Los Angeles County Calif*, 18, 443- 444.
- Cleveland, J., Montville, T.J., Nes, I.F. and Chikindas, M.L., 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol*, 71, 1-20.
- Cloutre, D.L. and Kandaswami, C. 2005. Grape seed extract. In: Coates P, Blackman M, Cragg G, et al., eds. *Encyclopedia of Dietary Supplements*. New York, NY: Marcel Dekker, 309-325.
- Code of Federal Regulations. 2002. Animals and animal products. Part 200 to end. Title 9, Part 424, subpart C 424.21. Washington, D.C.: Office of Federal Register, Natl. Archives and Records Admin.
- Conner, D.E. and Beuchat, L.R. 1984. Effects of essential oils from plants on growth of food spoilage yeasts. *J Food Sci*, 49, 429-434.
- Cooper, R., Morre, D.J., Morre, D.M. 2005. Medicinal benefits of green tea: part I. Review of noncancer health benefits. *J Alt Compl Med*, 11, 521- 528.
- Cortinas, L., Barroeta, A., Villaverde, C., Galobart, J., Guardiola, F. and Baucells, M.D. 2005. Influence of the dietary polyunsaturation level on chicken meat quality: Lipid oxidation. *Poul Sci*, 84, 48-55.
- Cos, P., De Bruyne, T., Hermans, N., Apers, S., Berghe, D.V. and Vlietinck, A.J. 2004. Proanthocyanadins in health care: Current and new trends. *Curr Med Chem*, 11, 1345-1359.

- Cox, S.D., Mann, C.M. and Markham, J.L. 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. *J Appl Microbiol*, 91, 492-497.
- Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinl Microbiol Rev*, 12, 564–582.
- Dai, J. and Mumper, R.J. 2010. Plant Phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15, 7313-7352.
- D'Aoust, J.Y. 1989. *Salmonella*. In: Doyle, M. P (Ed.), *Foodborne Bacterial Pathogens*, Marcel Dekker, New York, pp. 327-445.
- Das, D.N. 1962. Studies on the antibiotic activity of tea. *J Ind Chem Soc*, 39, 849-854.
- Davidson, P.M. and Naidu, A.S. 2000. Phytophenols. In: Naidu, A.S. (Ed.), *Natural Food Antimicrobial Systems*. Boca Raton, FL, USA, CRC Press LLC pp, pp. 265–294.
- Dawson, P.L., Janky, D.M., Dukes, M.G. Thompson, L.D. and Woodward, S.A. 1987. Effect of postmortem boning time during simulated commercial processing on the tenderness of broiler breast meat. *Poult Sci*, 66, 1331–1333.
- Deatherage, F.E. 1963. The effects of water and inorganic salts on tenderness. In: *Proceedings of Campbell's Soup Company, Meat Tenderness Symposium* (pp45-68). Camden, NJ.
- Debevere, J.M. 1989. The effect of sodium lactate on the shelf-life of vacuum-packed coarse liver pâté. *Fleischwirtsch*, 69, 223–224.
- De Vries, J. 2007. The obesity epidemic: medical and ethical considerations. *Sci Eng Ethics*, 13, 55-67.
- Desnuelle, P. and Savary, P. 1963. Specificities of lipases. *J Lipid Res*, 4, 369.
- Di Bonaventura, G., Piccolomini, R., Paludi, D., D'Orto, V., Vergara, A., Conter, M., et al. 2008. Influence of temperature on biofilm formation by *Listeria monocytogenes* on various foodcontact surfaces: relationship with motility and cell surface hydrophobicity. *J Appl Microbiol*, 104, 1552-1561.
- Diker, K.S. and Hascelik, G. 1994. The bactericidal activity of tea against *Helicobacter pylori*, *Lett Appl Microbiol*, 19, 299–300.
- Doyle, M.E., Mazzotta, A.S., Wang, T. Wiseman, D.W. and Scott, V.N. 2001. Heat resistance of *Listeria monocytogenes*. *J Food Prot*, 64, 410–429.
- Drewnowski, A. 1992. Sensory properties of fats and fat replacement. *Nutr Rev*, 50, 17-20.
- Elgayyar, M., Draughon, F.A., Golden, D.A. and Mount, J.R. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J Food Prot*, 64, 1019–1024.

Ettayebi, K., El Yamani, J. and Rossi-Hassani, B. 2000. Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. *FEMS Microbiol. Lett*, 183, 191–195.

Farber J.M. and Peterkin, P.I. 1991. *Listeria monocytogenes*, a food-borne pathogen. *Microbiol Rev*, 55, 476-511.

Faustman, C. and Cassens, R.G. 1990. The biochemical basis for discoloration in fresh meat: A Review. *J Muscle Foods*, 1, 217-243.

Food and Agricultural Organization. 1973. Toxicological evaluation of certain food additives with a review of general principles and specifications. 17<sup>th</sup> Report of Joint Food and Agriculture Organization of the United Nations/World Health Organization WHO Expert Committee on Food Additives. *WHO Technical Series No. 539*.

FDA. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate. *Federal Register*, 65, 3121–3123.

FDA/FSIS Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* among Selected Categories of Ready-to-Eat Foods. Department of Health and Human Services Food and Drug Administration/ United States Department of Agriculture Food Safety Inspection Service.

FDA. 2003. Direct food substances affirmed as generally recognized as safe. Code of Federal Regulations Title 21, Chapter I, Part 184.1754.

FDA, 2004. *Listeria monocytogenes*; petition to establish a regulatory limit docket no. 2003P-0574. *Federal Register*, 69, 29564 - 29565.

United States Department of Agriculture, Food Safety and Inspection Service. 2006. Basics of handling food safely.

Filus, K., Jankowski, J., Meller, Z. and Rotkiewicz, T. 1995. Meat quality and quantity of different hybrids of slaughter turkeys. Proceedings of XIIth European Symposium on the Quality of Poultry Meat, *Zaragoza*, pp 165-171.

Fostier, B., Turin, F., Chatelin, Y. and Cartier, P. 1993. Relationship between fat levels and flavor in minced beef, in *Proceedings of the 39<sup>th</sup> International Congress of Meat Science and Technology*, Calgary, File No. S7PO8.WP.

Frankel, E.N. 1993. In search for better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends Food Sci Technol*, 4, 220–225.

Frankel, E.N., Watrehouse, A.L. and Tussedre, P.L. 1995. Principle phenolic phytochemicals in selected california wines and their antioxidant activity in inhibiting oxidation of human low-density lipoprotein. *J Agri Food Chem*, 43, 890-894.

- Freese, E., Sheu, C.W. and Galliers, E. 1973. Function of lipophilic acids as antimicrobial food additives. *Nature*, 241, 321–325.
- Fujiki, H. 1999. Two stages of cancer prevention with green tea. *J Cancer Res Clin Oncol*, 125, 589 – 597
- Furiga, A., Lonvaud-Funel, A. and Badet, C. 2009. In vitro study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. *Food Chem*, 113, 1037–1040.
- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C.M. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *J Food Sci*, 73, 389-394.
- Gandhi, M. and Chikindas, M.L. 2007. *Listeria*: A foodborne pathogen that knows how to survive. *Int J Food Microbiol*, 113, 1–15.
- Gaze, J.E., Brown, G.D., Gaskell, D.E. and Banks, J.G. 1989. Heat resistance of *Listeria monocytogenes* in homogenates of chicken, beef steak and carrot. *Food Microbiol*, 6, 251–259.
- Geissman, T.A. 1963. Flavonoid compounds, tannins, lignins and related compounds, p. 265. In M. Florkin and E. H. Stotz (ed.), *Pyrrole pigments, isoprenoid compounds and phenolic plant constituents*, vol. 9. Elsevier, New York, N.Y.
- Gil, M.D., Buckley, J., Cayuela, J.M., Banon, S., Granados, M.V., Laencina, J. and Garrido, M.D. 2001. Estudio del efecto antioxidante de la vitamina E y de la catequina de te´ sobre carne de cerdo. In *Proceedings first Congreso Nacional de Ciencia y Tecnología de Alimentos* (pp. 276), 30 May–1 June 2001, Granada, Spain
- Glass, K.A., Granberg, D.A., Smith, A.L., Mc Namara, A.M., Hardin, M., Mattias, J., Ladwig, K. and Johnson, E.A. 2002. Inhibition of *Listeria monocytogenes* by sodium diacetate and sodium lactate on wieners and cooked bratwurst. *J Food Prot*, 65, 116–123.
- Glass, K.A. and Doyle, M.P. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl Environ Microbiol*, 55, 1565–1569.
- Gornowicz, E. and Dziadek, K. 2001. Variability in chemical composition of breast and thigh muscles depending on the broiler chickens (English abstr.). *Roczniki Naukowe Zootechniki*, 28, 89-100.
- Graham, H.N. 1992. Green tea composition, consumption, and polyphenol chemistry. *Preventive Medicine*, 21, 334–350.
- Gramza, A., Khokhar, S., Yoko, S., Gliszczynska-Swiglo, A., Hes, M. and Korczak, J. 2006. Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur J Lipid Sci Technol*, 108, 351–362.

- Griffin, P.M. and Tauxe, R.V. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev*, 13, 60–98.
- Grun, I.U., Ahn, J., Clarke, A.D. and Lorenzen, C.L. 2006. Reducing oxidation of meat. *Food Technol*, 60, 36–43.
- Guo, Q., Zhao, B., Shen, S., Hou, J. and Hu, J.X.W. 1999. ERS study on the structure antioxidant activity relationship of tea catechins and their epimers. *Biochem. Biophys Acta*, 1427, 13–23.
- Hamilton-Miller, J.M.T. 1995. Antimicrobial properties of tea (*Camellia sinensis*). *Antimicrob Agents and Chem*, 39, 2375-2377.
- Han, C. and Chen, J. 1995. The Screening of active anticarcinogenic ingredients in tea in Proceedings of 95 International Tea-Quality-Human Health Symposium, pp. 39 -41, 7-10 November, Shanghai, China
- Hao, Y.Y., Brackett, R.E. and Doyle, M. P. 1998. Inhibition of *Listeria monocytogenes* and *Aeromonas hydrophila* by plant extracts in refrigerated cooked beef. *J Food Prot*, 61, 307–312.
- Harris, 2007. Strawberries: Safe methods to store, preserve, and Enjoy. University of California. Division of Agriculture and natural resources, 8256, pp-15.
- Hashimoto, T., Kumazawa, S., Nanjo, F., Hara, Y. and Nakayama, T. 1999. Interaction of tea catechins with lipid bilayers investigated with liposome systems. *Biosci Biotechnol Biochem*, 63, 2252–2255.
- Hauben, K.J. ., Wuytack, E.Y., Sootjens, C.C.F. and Michiels, C.W. 1996. High pressure transient sensitization of *E. coli* to lysozyme and nisin by disruption of outer membrane permeability. *J Food Prot*, 59, 350-355.
- Helander, I.M., Alakomi, H.L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M. and von Wright, A. 1998. Charecterization of the action essential oil components on gram negative bacteria. *J Agri Food Chem*, 46, 3590-3595.
- Hennessy, T.W., Hedberg, C.W. and Slustker, L. 1996. A national outbreak of *Salmonella* Enteritidis infections from ice cream. *N Engl J Med*, 334, 1281-1286.
- Hintlian, C.B. and Hotchkiss, J.H. 1987. Comparative growth of spoilage and pathogenic organisms on modified atmosphere-packaged cooked beef. *J Food Prot*, 50, 218–223.
- Ho, J.L., Shands, K.N., Friedland, G., Eckind, P. and Fraser, D.W. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Arch Int Med*, 146, 520-524.

- Hoffman, C., Schweitzer, T.R., and Dalby, G. 1939. Fungistatic properties of the fatty acids and possible biochemical significance. *Food Res*, 4, 539.
- Hong, Y.H., Lim, G.O. and Song, K.B. 2009. Physical properties of *Gelidium corneum*-gelatin blend films containing grapefruit seed extract or green tea extract and its application in the packaging of pork loins. *J Food Sci*, 74, 6–10.
- Houtsma, P.C., de Wit, J.C. and Rombouts, F.M. 1993. Minimum inhibitory concentration (MIC) of sodium lactate for pathogens and spoilage organisms occurring in meat products. *Int J Food Microbiol*, 20, 247–257.
- Hu, X.F., Yang, X.Q. and Liu, K.X. 2001. Structure-activity relationship of tea polyphenols. Beijing, China: *Beijing Science Press*. pp 60–69
- Hu, M., Mc Clements, D.J. and Decker, E.A. 2004. Antioxidant activity of a proanthocyanidin-rich extract from grape seed in whey protein isolate stabilized algae oil-in-water emulsions. *J Agric and Food Chem*, 52, 5272–5276.
- Huang, D., Ou, B. and Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *J Agric Food Chem*, 53, 1841–1856.
- Hughes, E., Cofrades, S. and Troy, D.J. 1997. Effects of fat level, oat fiber and carrageenan on frankfurters formulated with 5, 12 and 30 % fat. *Meat Sci*, 45, 273-281.
- Hunter, D.R. and Segal, I.H. 1973. Effect of weak acids on amino acid transport by *Penicillium shrysogenum*: evidence for proton or charge gradient as the driving force. *J Bacteriol*, 113, 1184–1192.
- Hurst, A. 1977. Bacterial injury: A review. *Can J Microbiol*, 23, 935-944
- Hurst, A., and A. Hughes. 1978. Stability of ribosomes of *Staphylococcus aureus* S-6 sublethally heated in different buffers. *J Bacteriol*, 133, 564-568
- Hurst, A. 1981. Nisin. *Adv Appl Microbiol*, 27, 85-122.
- Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T. 1993. Bactericidal catechins damage the lipid bilayer. *Biochim Biophys Acta*, 1147, 132-136.
- Ismail, A.A. and Pierson, M.D. 1990. Effect of sodium nitrite and origanum oil on growth and toxin production of *Clostridium botulinum* in TYG broth and ground pork. *J Food Prot*, 53, 958–960.
- Jackson, T.C., Hardin, M.D. and Acuff, G.R. 1995. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures. *J Food Protect*, 59, 230–237.

- Jacob, J.K., Hakimuddin, F., Paliyath, G. and Fisher, H. 2008. Antioxidant and antiproliferative activity of polyphenols in novel high-polyphenol grape lines. *Food Res Int*, 41, 1-19.
- Jay, J.M., Loessner, M.J. and Golden, D.A. 2005. Modern Food Microbiology, Seventh Edition. New York: *Springer Science and Business media, Inc*, pp 301.
- Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res Int*, 36, 117–122.
- Jeremiah, L.E. 1978. A review of factors affecting meat quality. *Lacombe Res. Stn. Tech. Bull.* No. 1, Lacombe, Alb.
- Jo, C., Son, J.H., Son, C.B. and Byun, M.W. 2003. Functional properties of raw and cooked pork patties with added irradiated, freeze-dried green tea leaf extract powder during storage at 4 °C. *Meat Sci*, 64, 17–33.
- Jiang, A, Lu, C., Shu, A. and Wang, H. 1995. Application of tea flavonoids on healthy ice-cream' *China Tea*, 6, 12-13.
- Juven, B.J., Kanner, J., Schved, F. and Weisslowicz, H. 1994. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J Appl Bacteriol*, 76, 626–631.
- Kajiya, K., Hojo, H., Suzuki, M., Nanjo, F., Kumazawa, S. and Nakayama, T. 2004. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *J Agric Food Chem*, 52, 1514-1519.
- Karmali, M.A., Steele, B.T., Petric, M. and Lim, C. 1983. Sporadic cases of hemolytic uremic syndrome associated with fecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet I*, 619–620.
- Karori, S.M., Wachira, F.N., Wanyoko, J.K. and Ngure, R.M. 2007. Antioxidant capacity of different types of tea products. *Afr J Biotechnol*, 6, 2287–2296.
- Kawamura, J. and Takeo, T. 1989. Antibacterial activity of tea catechin to *Streptococcus mutans*. *J. Jpn. Soc. Food Sci. Technol*, 36, 463-467.
- Kar, P., Laight, D., Shaw, K.M. and Cummings, M.H. 2006. Flavonoid-rich grapeseed extracts: A new approach in high cardiovascular risk patients. *Int J Clin Prac*, 60, 1484–1492.
- Kerler, J. and Grosch, W. 1996. Odorants contributing to warmed over flavor (WOF) of refrigerated cooked beef. *J Food Sci*, 61, 1271-1274.
- Kim, T.J., Silva, J.L., Weng, W.L., Chen, W.W., Corbitt, M. and Jung, Y.S. 2009. Inactivation of *Enterobacter sakazakii* by water-soluble muscadine seed extracts. *Int J Food Microbiol*, 129, 295–299.

- King, N. J. and Whyte, R. 2006. Does it look cooked? A review of factors that influence cooked meat color. *J Food Sci*, 71, 31–40.
- Kinsella, J.E. 1990. Flavor perception and binding. *Inform*, 1, 215.
- Klontz, K.C., Timbo, B., Fein, S. and Levy, A. 1995. Prevalence of selected food consumption and preparation behaviors associated with increased risk of food-borne disease. *J Food Prot*, 58, 927-930.
- Kondo, K., Kurihara, M., Fukuhara, K. 2001. Mechanism of antioxidant effect of catechins. *Methods in Enzymol*, 335, 203-217.
- Komprda, T., Sarmanova, I., Zelenka, J., Bakaj, P., Fialova, M. and Fajmonova, E. 2002. Effect of sex and age on cholesterol and fatty acid content in turkey meat. *Arch Geflugelk*, 66, 263-273.
- Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci*, 43(Suppl.), 193-201.
- Lado, B.H. and Yousef, A.E. 2007. Characteristics of *Listeria monocytogenes* important to food processors. In E.T. Ryser, & E.H. Marth (Eds.), *Listeria, listeriosis and food safety* (pp. 157 - 214). CRC Press.
- Larson, A. E., Yu, R. R., Lee, O. A., Price, S., Haas, G. J. and Johnson, E. A. 1996. Antimicrobial activity of hop extracts against *Listeria monocytogenes* in media and in food. *Int J Food Microbiol*, 33, 195–207.
- Lau, D. W. and King, A. J. 2003. Pre- and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *J Agric Food Chem*, 51, 1602–1607.
- Lavelli, V., Vantaggi, C., Corey, M. and Kerr, W. 2010. Formulation of a dry green tea-apple product: study on antioxidant and color stability. *J Food Sci*, 75, 184 - 190.
- Lawrence, R.C., Fryer, T.F. and Reiter, B. 1967. The production and characterization of lipases from a micrococcus and a pseudomonad. *J Gen Microbiol*, 48, 401.
- Lawrie, R.A. 1998. The conversion of muscle to meat. pp 96–118 *In*: Lawrie's Meat Science. 6th ed. Woodhead Publishing Ltd., Cambridge, England.
- Lawrie, R.A. 2002. The eating quality of meat. *In*: Meat Science, 5th Edition. Pergamon Press. 173-176, 184-188.
- Lee M.J., Lambert, J.D., Prabhu, S., Meng, X.F. and Lu, H., Maliakal, P., Ho, C.T. and Yang, C.S. 2004. Delivery of tea polyphenols to the oral cavity by green tea levels and black tea extract. *Canc Epidemiol Biomark Prev*, 13, 132–137.

- Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *Int J Food Microbiol*, 55, 181–186.
- Leistner, L. and Gould, G. 2002. Hurdle Technologies: Combination treatments for food stability, safety and quality, Newyork: Kluwer Academic Publishers.
- Lesiów, T. 2006. Chemical composition of poultry meat. Handbook of Food Science, Technology and Engineering. Edited by Y. Hui, CRC Taylor & Francis Group, Boca Raton. London, New York, 1, 32.
- Lin, J. and Liang, Y. 2000. Cancer chemoprevention by tea polyphenols. *Proceedings of Natl Sci Counc ROC (B)* 24, 1–13.
- Loncin M. 1975. Basic principles of moisture equilibria. In: Goldblith SA., Rey L, Rothmayr WW, editors. Freeze drying and advanced food technology. New York: Academic Press. p 599–617.
- Lugasi, A., Dworschak, E. and Hovari, J. 1995. Characterization of scavenging activity of natural polyphenols by chemiluminescence technique. Fed. European Chem. Soc. Proceed. of Euro Food Chem. VIII, Vienna, Austria, 3, 639-643.
- Lunden, J., Autio, T., Markkula, A., Hellstrom, S. and Korkeala, H. 2003. Adaptive and cross-adaptive responses of persistent and non-persistent *Listeria monocytogenes* strains to disinfectants. *Int J Food Microbiol*, 82, 265-272.
- Maier, T., Schieber, A., Kammerer, D.R. and Carle, R. 2009. Residues of grape (*Vitis vinifera*) seed oil production as a valuable source of phenolic antioxidants. *Food Chem*, 112, 551 –559.
- Majo, D.D., Guardia, M.L., Giammanco, S., Neve, L.L. and Giammanco, M. 2008. The antioxidant capacity of red wine in relationship with its polyphenolic constituents. *Food Chem*, 111, 45–49.
- Martinez, L., Cilla, I., Beltran, J.A. and Roncales, P. 2006. Antioxidant effect of rosemary, borage, green tea, pu-erh tea and ascorbic acid on fresh pork sausages packaged in a modified atmosphere: influence of the presence of sodium chloride. *J Sci Food Agri*, 86, 1298–1307.
- Marusich, W.L., De Ritter, E., Ogrinz, E.F., Keating, J., Mitrovic, M. and Bunnell, R.H. 1975. Effect of supplemental vitamin E in control of rancidity in poultry meat. Animal Health Research Department and Product Development Department, Hoffmann-La Roche Inc., Nutley, NJ.
- Mason, T.L. and Wasserman, B.P. 1987. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. *Phytochem*, 26, 2197–2202.
- Matthews, C.M. 2010. Steep your genes in health: drink tea. *Proceedings (Baylor University Medical Centre)*, 23, 142–144.

- Mbandi, E. and Shelef, L. A. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. *J Food Prot*, 64, 640–644.
- Mbandi, E. and Shelef, L. A. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int J Food Microbiol*, 76, 191-198.
- Mbata T.I., Debiao L. and Saikia, A. 2006. Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*. *Internet J Microbiol*, 7, 1571-1573.
- McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B. and Buckley, D.J. 2001a. Assessment of the antioxidant potential of natural food and plant extracts in fresh and previously frozen pork patties. *Meat Sci*, 57, 177–184.
- McCarthy, T.L., Kerry, J.P., Kerry, J.F., Lynch, P.B. and Buckley, D.J. 2001b. Evaluation of the antioxidant potential of natural food/plant extracts as compared with synthetic antioxidants and vitamin E in raw and cooked pork patties. *Meat Sci*, 57, 45–52.
- Meilgaard, M.C. 1991. Testing for sensory threshold of added substances. *J Am Soc Brew Chem*, 49, 128-135.
- Mela, D.J. 1990. The basis of dietary fat preference. *Trends Food Sci. Technol*, 1, 55-78.
- Mielnik, M.B., Olsen, E., Vogt, G., Adeline, D. and Skrede, G. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. *Food Sci Technol*, 39, 191–198.
- Mozafari, M.R., Flanagan, J., Matia-Merino, L., Awati, A., Omri, A. and Suntres, Z.E. 2006. Recent trends in the lipid-based nanoencapsulation of antioxidants and their role in foods. *J Sci Food Agric*, 86, 2038–2045.
- Mildner-Szkudlarz, S., Zawirska-Wojtasiak, W., Obuchowski, W. and Golinski, M. 2009. Evaluation of antioxidant activity of green tea extract and its effect on the biscuits lipid fraction oxidative stability. *J Food Sci*, 74, 362–370.
- Mielnik, M.B., Olsen, E., Vogt, G., Adeline, D. and Skrede, G. 2006. Grape seed extract as antioxidant in cooked, cold stored turkey meat. *Food Sci Technol*, 39, 191–198.
- Miki, U., Hideyuki, Y. and Masaki, S. 1991. Effect of tea catechins in halitosis and their application in chewing gum. *Nippon Shokuhin Kogyo Gakkaishi*, 38, 1098-1102
- Miller, R.K., Acuff, G.R. 1994. Sodium lactates affects pathogens in cooked beef. *J Food Sci*, 9, 15–19.

- Mishu, B., Koehler, J., Lee, L.A., Rodrigue, D., Brenner, H., Blake, P. and Tauxe, R. 1994. Outbreaks of *Salmonella* Enteridis infections in the United States, 1985-1991. *J Infect Dis*, 169, 542-547.
- Mitsumoto, M., O'Grady, M.N., Kerry, J.P. and Buckley, D.J. 2005. Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. *Meat Sci*, 69, 773-779.
- Murphy, R.Y., Hanson, R.E., Feze, N., Johnson, N.R., Scott, L.L., Duncan, L.K. 2005a. Eradicating *Listeria monocytogenes* from fully cooked franks by using an integrated pasteurization-packaging system. *J Food Prot*, 68, 508-511.
- Murphy, R.Y., Hanson, R.E., Johnson, N.R., Scott, L.L., Feze, N. and Chappa, K. 2005b. Combining antimicrobials and steam treatment in a vacuum packaging system to control *Listeria monocytogenes* on ready-to-eat frankfurters. *J Food Sci*, 70, 135-140.
- Murphy, R.Y., Hanson, R.E., Johnson, N.R., Chappa, K. and Berang, M.E. 2006. Combining organic acid treatment with steam pasteurization to eliminate *Listeria monocytogenes* on fully cooked frankfurters. *J Food Prot*, 69, 47-52.
- Murano, E.A. and Rust, R.E. 1995. General microbial profile of low fat frankfurters formulated with sodium lactate and a texture modifier. *J Food Qual*, 18, 313-323.
- Nashif, S.A. and Nelson, F.E. 1953. The lipase of *Pseudomonas fragi*. II. Factors affecting lipase production. *J Dairy Sci*. 36, 471.
- Negro, C., Tomais, L. and Miceli, A. 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresour Technol*, 87, 41-44.
- Nerbrink, E., Borch, E., Blom H. and Nesbakken, T. 1999. A model based on absorbance data on the growth rate of *Listeria monocytogenes* and including the effects of pH, NaCl, Na-lactate and Na-acetate. *Int J Food Microbiol* 47, 99-109.
- Mbandi, E. and Shelef, L.A. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. *J Food Prot*, 64, 315-320.
- McMeekin, T.A., Presser, K., Ratkowsky, D., Ross, T., Salter, M. and S. Tienungoon. 2000. Quantifying the hurdle concept by modeling the bacterial growth/no growth interface. *Int. J. Food Microbiol*, 55, 93-98.
- Montville, T.J and Matthews, K.R. 2005. Food Microbiology: an introduction (pp.21 -22) Washington DC, ASM press.

- Modi, K.D., Chikindas, M.L. and Montville, T.J. 2000. Sensitivity of nisin-resistant *Listeria monocytogenes* to heat and the synergistic action of heat and nisin. *Lett Appl Microbiol*, 30, 249–253.
- Monteleone, E., Condelli, N., Dinnella, C. and Bertuccioli, M. 2004. Prediction of perceived astringency induced by phenolic compounds. *Food Qual Preference*, 15, 761–769
- Murry, E.G.D., Webb, R.A. and Swann, B.R. 1926. A disease of rabbit characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp). *J Pathol Bacteriol*, 29, 407-439.
- Naidu, A.S. 2000. Natural food antimicrobial systems. In Juneja, L.R., Okubo, T. and Hung, P (Eds.), *Catechins* (pp 382). Florida: CRC Press LLC.
- Nelson, F.E. 1944. Factors which influence the growth of heat treated bacteria: I. A Comparison of four agar media. *J Bacteriol*, 45, 395-403.
- Nilsson, L., Chen, Y., Chikindas, M.L., Huss, H.H., Gram, L. and Montville, T.J. 2000. Carbon dioxide and nisin act synergistically on *Listeria monocytogenes*. *Appl Environ Microbiol*, 66, 769–774.
- Nissen, L.R., Byrne, D.V., Bertelsen, G. and Skibsted, L.H. 2004. The antioxidative activity of plant extracts in cooked pork patties as evaluated by descriptive sensory profiling and chemical analysis. *Meat Sci*, 68, 485–495.
- Oh, D., Lee, M.K. and Park, B.K. 1999. Antimicrobial activities of commercially available tea on the harmful foodborne organisms. *J Korean Soc Food and Nutr*, 28, 100-106.
- Olsson, U., C. Hertzman and Tornberg, E. 1994. The influence of low temperature, type of muscle and electrical stimulation on the course of rigor mortis, aging and tenderness of beef muscles. *Meat Sci*, 37, 115-131
- Otake, S., Makimura, M., Kuroki, T., Nishihara, Y. and Hirasawa, M. 1991. Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Res*, 25, 438-443.
- Over, K.F., Hettiarachchy, N.S., Johnson, M.G. and Davis, B. 2009. Effect of organic acids and plant extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium in broth culture model and chicken meat systems. *J Food Sci*, 74, M515-521.
- Papadopoulos, L.S., Miller, R.K., Acuff, G.R., Lucia, L.M., Vanderzant, C. and Cross, H.R. 1991. Consumer and trained sensory comparisons of cooked beef top rounds treated with sodium lactate. *J Food Sci*, 56, 1141–1146.
- Palumbo, S.A., Pickard, A. and Call, J.E. 1997. Population changes and verotoxin production of enterohemorrhagic *E. coli* strains inoculated in milk and ground beef held at low temperatures. *J Food Prot*, 60, 746–775.

- Pastrana-Bonilla, E., Akoh, C.C., Sellappan, S. and Krewer, G. 2003. Phenolic content and antioxidant capacity of muscadine grapes. *J Agric Food Chem*, 51, 4497–4503.
- Pazos, M., Gallardo, J.M., Torres, J.L. and Medina, I. 2004. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chem*, 92, 547–557.
- Peryam, D.R., Pilgrim, F.J. and Peterson, M.S. (Eds). 1954. Food acceptance testing methodology. Washington, DC: National Academy of Sciences. *National Research Council*
- Pfenninger, H.B. 1979. “Methods of quality control in brewing,” *Schweizer Brauerei-Rundschau*, 90, 121.
- Porto, A.C.S., Franco, D.G. M., Sant’Anna, E.S., Call, J.E., Piva, A. and Luchansky, J.B. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with or without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10 °C. *J Food Prot*, 65, 308–315.
- Pflug, I.J. 1997. Evaluating a ground beef patty cooking process using the general method of process calculation. *J Food Prot*, 60, 1215-1223.
- Qiao, M., Fletcher, D.L., Northcutt, J.K. and Smith, D.P. 2002. The relationship between raw broiler breast meat color and composition. *Poult Sci*, 81, 422-427.
- Rababah, T., Hettiarachchy, N.S., Eswaranandam, S., Meullenet, J.F. and Davis, B. 2005. Sensory evaluation of irradiated and nonirradiated poultry breast meat infused with plant extracts. *J Food Sci*, 70, 228–235.
- Rababah, T., Hettiarachchy, N.S., Horax, R., Cho, M.J., Davis, B. and Dickson, J. 2006. Thiobarbituric acid reactive substances and volatile compounds in chicken breast meat infused with plant extracts and subjected to electron beam irradiation. *Poult Sci*, 85, 1107–1113.
- Rajkowski, K.T. and Marmer, B.S. 1995. Growth of *Escherichia coli* O157:H7 at fluctuating incubation temperatures. *J Food Prot*, 58, 1307–1313.
- Rall, T.W. 1990. In: A.G. Gilman; T.W. Rall; A.S. Nies and P. Taylor (eds.), Goodman and Gilman’s the pharmacological basis of therapeutics, 8th ed.,p. 618-637. Pergamon, New York.
- Ralovich, B. 1992. Data for the properties of *Listeria* strains (a review). *Acta Microbiol Hung*, 39, 105–132.
- Rein, D., Paglieroni, T.G., Pearson, D.A., Wun, T., Schmitz, H.H., Gosselin, R. and Keen, C.L. 2000. Cocoa and wine polyphenols modulate platelet activation and function. *J Nutr*, 130, 2120–2126.

- Rhodes, P.L., Mitchell, J.W., Wilson, M.W. and Melton, L.D. 2006. Antilisterial activity of grape juice and grape extracts derived from *Vitis vinifera* variety Ribier. *Int J Food Microbiol*, 107, 281–286.
- Rocourt, J., Cossart, P., 1997. *Listeria monocytogenes*. In: Doyle, M.P., Buechat, L.R., Montville, T.J. (Eds.), *Food Microbiology - Fundamentals and Frontiers*. American Society for Microbiology (ASM) Press, Washington DC, pp. 337–352.
- Rocourt, J. and Buchrieser, C. 2007. The genus *Listeria* and *Listeria monocytogenes*: phylogenetic position. In E.T. Ryser, and E.H. Marth (Eds.), *Listeria, listeriosis and food safety* (pp. 1 - 20). CRC Press.
- Rojas, M.C. and Brewer, M.S. 2007. Effect of natural antioxidants on oxidative stability of cooked, refrigerated beef and pork. *J Food Sci*, 72, 282–288.
- Rosenblat, M., Volkova, N., Coleman, R., Almagor, Y. and Aviram, M. 2008. Antiatherogenicity of extra virgin olive oil and its enrichment with green tea polyphenols in the atherosclerotic apolipoprotein-E-deficient mice: enhanced macrophage cholesterol efflux. *J Nutr Biochem*, 19, 514–523.
- Rotava, R., Zanella, I., da Silva, L. P., Manfron, M. P., Ceron, C. S. and Alves, N. P. 2009. Antibacterial, antioxidant and tanning activity of grape by-product. *Ciencia. Rural*, 39, 941–944.
- Roychowdhury, S., Wolf, G., Keilhoff, G., Bagchi, D., and Horn, T. 2001a. Grape seed proanthocyanidin extract (GSPE) protects astroglia against nitrosative/oxidative stress. *J Neurochem*, 77, 38.
- Roychowdhury, S., Wolf, G., Keilhoff, G., Bagchi, D. and Horn, T. 2001b. Protection of primary glial cells by grape seed proanthocyanidin extract against nitrosative/oxidative stress. *Nitric Oxide—Biol Chem*, 5, 137–149.
- Ruhr, E. and Sahl, H.G. 1985. Mode of action of peptide antibiotic nisin and influence on the membrane potential of whole cells and on cytoplasmic and artificial membrane vesicles. *Antimicrob Agents Chemother*, 27, 841-845
- Russell, S.M. 2003. The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs. *Poult Sci*, 82, 158–162.
- Rybka-Rodgers, S. 2001. Improvement of food safety design of cook-chill foods. *Food Res Int*, 34, 449-455.
- Sakanaka, S., Kim, M., Taniguchi, M. and Yamamoto, T. 1989. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric Biol Chem*, 53, 2307-2311.

Salmond, C.V., Kroll, R.G. and Booth, I.R. 1984. The effects of food preservatives on pH homeostasis in *Escherichia coli*. *J Gen Microbiol*, 130, 2845–2850.

Samelis, J., Bedie, G.K., Sofos, J.N., Belk, K.E., Scanga, J.A. and Smith, G.C. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4 °C in vacuum packages. *J Food Prot*, 65, 299–307.

Sanderson, G.W. 1972. The chemistry of tea and tea manufacturing' in structural and functional aspects of phytochemistry, (Runeckles, V.C., ed), pp. 247 - 316, Academic Press, NY.

Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M. and Ochi, H. 1996. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. *J Agric Food Chem*, 44, 37–41.

Scherm, H., Savelle, A.T. and Law, S.E. 2007. Effect of electrostatic spray parameters on the viability of two bacterial biocontrol agents and their deposition on blueberry flower stigmas. *Biocont Sci Technol*, 17, 285-293.

Schwartz, B., Ciesielski, C.A., Broome, C.V., Gaventa, S., Brown, G.R., Gellin, B.G., Hightower, A.W. and L. Mascola. 1998. Association of sporadic listeriosis with consumption of uncooked hot dogs and under cooked chicken. *Lancet II*, 779-782.

Seelinger, H.P.R. and Jones D. 1986. Genus *Listeria*. In: Sneath PHA, Mair NS, Sharpe ME, G. HJ, editors. Bergey's manual of systemic bacteriology. Baltimore: Williams and Wilkins. pp 1235–1245.

Seman, J.L., Borger, A.C., Meyer, J.D., Hall, P.A., Milkowski, A.L. 2002. Modeling of the growth of *Listeria monocytogenes* in cured ready-to-eat meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. *J Food Prot*, 65, 651–658.

Shaker, E.S. 2006. Antioxidative effect of extracts from red grape seed and peel on lipid oxidation in oils of sunflower. *Lebensmittel– Wissenschaft und–Technologie*, 39, 883–892.

Shan, B., Cai, Y.Z., Brooks, J.D. and Corke, H. 2007. Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): Activity against foodborne pathogenic bacteria. *J Agric Food Chem*, 55, 5484–5490.

Shanmuganayagam, D., Beahm, M.R., Osman, H.E., Krueger, C.G., Reed, J.D. and Folts, J.D. 2002. Grape seed and grape skin extracts elicit a greater antiplatelet effect when used in combination than when used individually in dogs and humans. *J Nutr*, 132, 3592–3598.

Shee, A.K. 2010. Studies on the current antibacterial activity potential of commonly used food preservatives. *Int J Eng Technol*, 2, 264-269.

Shelef, L.A., 1984. Antimicrobial effects of spices. *J Food Saf*, 6, 29–44.

- Shelef, L.A. and Addala, L. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J Food Saf*, 14, 103-115
- Sheldon, B.W. 1984. Effect of dietary tocopherol on the oxidative stability of turkey meat. *Poul Sci*, 63, 673-681
- Shi, J., Yu, J., Pohorly, J.E. and Kakuda, Y. 2003. Polyphenolics in grape seeds-biochemistry and functionality. *J Med Food*, 6, 291-299.
- Shimamura, T., Zhao, W.H. and Hu, Z. 2007. Mechanism of action and potential for use of tea catechin as an anti-infective agent. *Anti-Inf Agents in Med Chem*, 6, 57-62.
- Shoko, T., Soichi, T., Megumi, M.M., Eri, F., Jun, K. and Michiko, W. 1999. Isolation and identification of an antibacterial compound from grape and its application to foods. *Nippon Nogeikagaku Kaishi*, 73, 125-128.
- Si, W., Gong, J., Tsao, R., Kalab, M., Yang, R. and Yin, Y. 2006. Bioassay-guided purification and identification of antimicrobial components in Chinese green tea extract, *J Chrom*, 1125, 204-210.
- Sivarooban, T., Hettiarachchy N.S. and Johnson, M.G. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10 °C. *J Food Prot*, 70, 1017-1020.
- Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008a. Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extract. *J Food Prot*, 71, 2105-2109.
- Sivarooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008b. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Res Intl*, 41, 781-785.
- Soobrattee, M.A., Neergheena, V. S., Luximon-Rammaa, A., Aruomab, O. I. and Bahoruna, T. 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*, 57, 200-213.
- Spranger, I., Sun, B., Mateus, A.M., de Freitas, V. and Ricardo-da-Silva, J.M. 2008. Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. *Food Chem*, 108, 519-532.
- Stewart, J.A., Mullen, W. and Crozier, A. 2004. On-line high performance liquid chromatography of the antioxidant of phenolic in green and black tea. *Mol Nutr Food Res*, 49, 52-60.

- Su, P., Henriksson, A., Nilsson, C. and Mitchell. 2008. Synergistic effect of green tea extract and probiotics on the pathogenic bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*. *W J Microbiol Biotechnol*, 24, 1837–1842.
- Tagurt, T., Tanaka, T. and Kouno, I. 2004. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. *Biol Pharma Bulletin*, 27, 1965–1969.
- Theivendran, S., Hettiarachchy, N.S., and Johnson, M.G. 2006. Inhibition of *Listeria monocytogenes* by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *J Food Sci*, 71, 39-44.
- Tilden Jr., J., Young, W., McNamara, A.M., Custer, C., Boesel, B., Lambert-Fair, M.A., Majkowski, J., Vugia, D., Werner, S.B., Hollingsworth, J., and Morris, J.G. 1996. A new route of transmission for *Escherichia coli*: Infection from dry fermented salami. *Am J Public Health*, 86, 1142–1145.
- Sklan, D., Tenne, Z. and Budowski, P. 1983. Simultaneous lipolytic and oxidative changes in turkey meat stored at different temperatures. *J Food Agric*, 34, 93-99.
- Sklan, D. and Tenne, Z. 1984. Changes in lipid fractions and bacteriological counts in chilled broiler meat. *Poult Sci*, 63, 76-81.
- Sommers, C.H., Fan, X., Niemira, B.A. and Sokorai, K. 2003. Radiation (gamma) resistance and post-irradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. *J Food Prot*, 66, 2051–2056.
- Smith, L.T. 1996. Role of osmolytes in adaptation of osmotically stressed and chill stressed *Listeria monocytogenes* grown in liquid media and on processed meat surfaces. *Appl Environ Microbiol*, 62, 3088–3093.
- Stecchini, M.L., Giavedoni, P., Sarais, I. and Lericci, C.R. 1993. Antimicrobial activity of Maillard reaction products against *Aeromonas hydrophila*. *Int J Food Sci*, 5, 147–150.
- Stekelenburg, F.K., Kant-Muermans, M.L.T. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int J Food Microbiol*, 66, 197– 203.
- Stone, H. and Sidel, J.L. 2004. Sensory evaluation practices, third edition. Redwood city, CA: Tragon corporation. 377 pp.
- Stumbo, C.R. 1973. Thermobacteriology in food processing, 2<sup>nd</sup> ed. Academic Press, New York.
- Swaminathan, B. and Gerner-Smidt, P. 2007. The epidemiology of human listeriosis. *Microbial Infect*, 9, 1236-1243.

- Sullivan C.M., Lynch, A.M., Lynch, P.B., Buckley, D.J. and Kerry, J.P. 2004. Assessment of the antioxidant potential of food ingredients in fresh, previously frozen and cooked chicken patties. *Int J Poult Sci*, 3, 337-344.
- Tan, W. and Shelef, L.A. 2002. Effects of sodium chloride and lactates on chemical and microbiological changes in refrigerated and frozen fresh ground pork. *Meat Sci*, 62, 27–32.
- Tang, S.Z., Kerry, J.P., Sheehan, D. and Buckley, D.J. and Morrissey, P.A. 2000. Dietary tea catechins and iron-reduced lipid oxidation in chicken meat, liver and heart. *Meat Sci*, 56, 285-290
- Tang, S., Kerry, J.P., Sheehan, D., Buckley, D.J. and Morrissey, P.A. 2001. Antioxidative effect of added tea catechins on susceptibility of cooked red meat, poultry and fish patties to lipid oxidation. *Food Res Int*, 34, 651–657.
- Taylor, P.W., Hamilton-Miller, J.M.T. and Stapleton, P.D. 2005. Antimicrobial properties of green tea catechins. *Food Sci Technol Bulletin*, 2, 71–81.
- Teo, Y.L., Raynor, T.J., Ellajosyula, K.R., and Knabel, S.J. 1996. Synergistic effect of temperature and high pH on the destruction of *Salmonella enteritidis* and *Escherichia coli* O157:H7. *J Food Protect*, 59, 1023–1030.
- Toda, M., Okubo, S., Hara, Y. and Shinamura, T. 1991. Antibacterial and bactericidal activities of tea extracts and catechins against methicillin-resistant *Staphylococcus aureus*. *Jpn J Bacteriol*, 46, 839-845.
- Toda, M., Okubo, S., Ikigay, H., Suzuki, T., Suzuki, Y., Hara, Y. and Shinamura, T. 1992. The protective activity of tea catechins against experimental infection by *Vibrio cholerae* O1. *Microbiol. Immunol*, 36, 999-1001.
- Tomlins, R.I., Pierson, M.D., and Ordal, Z.J. 1971. Effect of thermal injury on the TCA cycle enzymes of *Staphylococcus aureus* MF 31 and *Salmonella Typhimurium* 7136. *Can J Microbiol*, 17, 759 – 765.
- Tompkin, R.B., 2002. Control of *Listeria monocytogenes* in the food processing environment. *J Food Prot*, 65, 709-725.
- Tsigarida, E., Skandamis, P., Nychas, G.J. 2000. Behaviour of *Listeria monocytogenes* and autochthonous flora on meat stored under aerobic, vacuum and modified atmosphere packaging conditions with or without the presence of oregano essential oil at 5 °C. *J Appl Microbiol*, 89, 901–909.
- Tsuchiya, H., Sato, M., Miyazaki, T., Fujiwara, S., Tanigaki, S., Ohyama, M., Tanaka, T. and Linuma, M. 1996. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J Ethnopharmacol*, 50, 27-34.

- USDA/FSIS, 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule, 9 CFR Part 430. *Fed Regist* 68, 34208-34254.
- Vaara, M. 1992. Agents that increase the permeability of the outer membrane. *Microbiol Rev*, 56, 395-411.
- Vanee, K. 2006. Food Packaging: New Technology. Handbook of Food Science, Technology and Engineering, 3, Hui, Y. H. (ed), Taylor and Francis, New York.
- Vaquero, M.J.R., Alberto M.R., and Nadra, M.C.A. 2007. Influence of phenolic compounds from wines on the growth of *Listeria monocytogenes*. *Food Cont*, 18, 587–593.
- Vijay, J., Hwang, C.A. and Freidman, M. 2010. Thermal Inactivation and Post-treatment growth during storage of multiple *Salmonella* serotypes in ground beef as affected by sodium lactate and oregano oil. *J Food Sci*, 75, 1-6.
- Vinson, J.A., Dabbagh, Y.A., Serry, M.M. and Jang, J. 1995. Plant flavonoids, especially tea flavonoids, are powerful antioxidants using an in vitro oxidation model for heart disease. *J Agric Food Chem*, 43, 2800 - 2802
- Wang, H. 2000. Tea flavonoids: their functions, utilisation and analysis. *Trends in Food Sci Technol*, 11, 152 – 160.
- Wang, R. and Zhou, W. 2004. Stability of tea catechins in the breadmaking process. *J Agric Food Chem*, 52, 8224–8229.
- Warriner, K. and Namvar, A. 2009. What is the hysteria with Listeria? *Trends in Food Sci Technol*, 20, 245-254.
- Weber, H.A., Hodges, A.E., Guthrie, J.R., O'Brien, B.M., Robaugh, D., Clark, A.P., et al. 2007. Comparison of proanthocyanidins in commercial antioxidants: Grape seed and pine bark extracts. *J Agric Food Chem*, 55, 148–156.
- Wei, He., Hu, X., Zhao, L., Liao, X., Zhang, T., Zhang, M. and Wu, J. 2009. Evaluation of Chinese tea by the electronic tongue: Correlation with sensory properties and classification according to geographical origin and grade level. *Food Res Int*, 42, 1462-1467.
- Wills, E. D. 1965. Lipase. *Adv. lipid Res.* 3, 197.
- Wiseman, S.A., Balentine, D.A. and Frei, B. 1997. Antioxidants in Tea. *Crit Rev Food Sci Nutr* 37, 705 – 718.
- Wu, C.D. and Wei, G.X. 2002. Tea as a functional food for oral health. *Nutr*, 18, 443–444.
- Wu, Y., Chen, Z.X., Li, X.X., and Li, M. 2009. Effect of tea polyphenols on the retrogradation of rice starch. *Food Res Int*, 42, 221–225.

- Xia, E.Q., Deng, G.F., Guo, Y.J. and Li, H.B. 2010. Biological activities of polyphenols from grapes – review. *Int J Mol Sci*, 11, 622-646.
- Yang, X.Q., Wang, Y.F. and Xu, F. 1995. Natural antioxidant tea polyphenols application on oil and food: study on inhibiting the deterioration of salad oil and instant noodles. *J University of Agriculture of Zhejiang*, 21, 513-518
- Yasuda, H. 1992. Deodorant effect of tea catechins and their application, *Shokuhin Kogyo*, 35, 28-33
- Yeo, S.G., Ahn, C.W., Kim, I.S., Park, Y.B., Park, Y.H. and Kim, S.B. 1995. Antimicrobial effect of tea extracts from green tea, oolong tea and black tea. *J Kor Soc Food and Nutr*, 24, 293-298.
- Yilmaz, Y. and Toledo, R.T. 2004. Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J Agric Food Chem*, 52, 255-260.
- Yilmaz, Y. 2006. Novel uses of catechins in foods. *Trends in Food Sci Technol*, 17, 64-71.
- Yoda, Y., Hu, Z.Q., Zhao, W.H. and Shimamura, T. 2004. Different susceptibilities of *Staphylococcus* and Gram-negative rods to epigallocatechin gallate. *J Inf Chemother*, 10, 55-58
- Zhang, J. and Kashket, S. 1998. Inhibition of salivary amylase by black and green teas and their effects on the intraoral hydrolysis of starch. *Caries Res*, 32, 233-238
- Zhang, L., Gu, F.X., Chan, J.M., Wang, A.Z., Langer, R.S. and Farokhzad, O.C. 2008. Nanoparticles in medicine: therapeutic applications and developments. *Clinl Pharm Therap*, 83, 761-769.

## CHAPTER III

### EFFECT OF POTASSIUM LACTATE AND SODIUM DIACETATE COMBINATION TO INHIBIT *LISTERIA MONOCYTOGENES* IN LOW AND HIGH FAT CHICKEN AND TURKEY HOTDOG MODEL SYSTEMS

#### ABSTRACT

Effect of potassium lactate (PL) and sodium diacetate (SD) combinations at varying levels were evaluated in low (5 %) and high (20 %) fat chicken and turkey hotdog model systems. All samples were surface inoculated with *Listeria monocytogenes* [approximately 4.62 log colony forming units cfu/g (cfu/g)], vacuum packed, and stored at 4 °C for twenty-eight days to determine the effective combination of PL and SD and the effect of fat content on the growth inhibition of *L. monocytogenes*. In chicken hotdog samples, maximum growth inhibitions (3.42 log cfu/g) were observed in low fat samples formulated with 3.0 % PL and 0.15 % SD. In turkey hotdog samples, maximum growth inhibitions (3.3 log cfu/g) were observed in low fat samples formulated with 3.0 % PL and 0.2 % SD in turkey. Effective combination levels determined in low and high fat chicken were 3.0% PL and 0.15% SD, whereas in low and high fat turkey, the effective levels were 3.0% PL and 0.20% SD. Overall, fat content had a significant effect ( $P < 0.05$ ) on growth inhibition as indicated by higher inhibitions in low fat chicken and turkey hotdogs than high fat samples. These results demonstrate that commercial usage levels of PL (2.0 %) and SD (0.15 %) alone are not sufficient to control *L. monocytogenes* in case of pathogen contamination.

#### INTRODUCTION

*Listeria monocytogenes* (*L. monocytogenes*) is the most common post-processing bacterial contaminant in ready-to-eat (RTE) meat and poultry products and has elicited intense concerns from consumers and processors due to recurrent outbreaks resulting in associated

product recalls (Rocourt et al., 2003; Olsen et al., 2005; Gottlieb et al., 2006; Lianou and Sofos, 2007). Among the RTE foods contaminated by *L. monocytogenes*, deli meats and non-reheated hotdogs pose “very high risk” per serving risk of illness/death (FDA, 2003). As a result, the U.S. Dept. of Agriculture (USDA) and Food and Drug administration (FDA) maintains a “zero tolerance” (no detectable levels permitted) policy for *L. monocytogenes* in RTE products (FDA, 2001; Klontz et al., 2008).

Based on the public health significance and treatment costs, there is a need to develop effective intervention strategies that will inhibit or kill the pathogens and improve the microbiological quality of the meat (CDC, 2002; Corbo et al., 2009). Control measures for safety of RTE poultry products should include reducing the risk of contamination as well as inhibiting the growth of pathogens during handling and storage. Conventional chemical antimicrobials such as lactates and diacetates act as bacteriostatic agents against foodborne pathogens including *L. monocytogenes* in meat and poultry products.

Potassium lactate (PL) is a clear syrupy liquid derived from lactic acid and acts as a bacteriostatic agent by extending the lag phase or dormant phase of pathogens and thereby prolonging the shelf life of food products (Blom et al., 1997; Stekelenburg and Kant-Muermans, 2001). The specific mechanisms of actions of lactates are reducing the water activity ( $a_w$ ) of the product (Brewer et al., 1991; Chen and Shelef, 1992; Miller and Acuff, 1994) and intracellular acidification (Hunter and Segal, 1973). Sodium diacetate (SD) is bactericidal in action against *L. monocytogenes* by lowering the intracellular pH and significantly inhibiting the growth of the initial bacterial load (Shelef and Addala, 1994). Both PL and SD are FDA approved and classified as GRAS (Generally Recognized as Safe) ingredients in RTE meat and poultry products (FDA, 2000). Maximum permissible levels of lactates (60 % lactate solution) and

diacetates used in the meat formulations are 4.8 % and 0.25% respectively, based on the batch weight of total formulation (Keeton, 2001). However, higher concentration levels of lactates (> 3 %) affect sensory properties (Knight et al., 2007; Nunez et al., 2004). The maximum level for sodium diacetate from a flavor perspective is 0.1 to 0.15%, whereas its inhibitory effects become apparent from 0.125% (Shelef and Addala, 1994; Weber, 1997). At 0.20 % concentration, it has a negative effect on odor and taste of the product (Stekelenburg and Kant-Muermans, 2001). Combination of these two antimicrobials has demonstrated enhanced inhibition of the growth of *L. monocytogenes* on RTE meats during long-term refrigerated storage than when used alone (Mbandi and Shelef, 2001, 2002; Seman et al., 2002; Sommers et al., 2003; Nunez et al., 2004; Knight and Castillo, 2007).

Antimicrobial activity of any preservative depends on their hydrophilic and hydrophobic properties i.e. solubility in water and fat, distribution in the model system, fat content, and pH and temperature (Gooding et al., 1955, Glass and Doyle, 1989). Effective combinations of lactates and diacetates vary from one product to another as influenced by differences in meat matrices, formulations (types of meat, moisture and fat content, water activity, pH, salt, and nitrite levels), storage temperature, and packaging conditions (Buchanan et al., 1993; Houstma et al., 1996 a, b; Hu and Shelef, 1996; Seman et al., 2002). In addition, in biphasic foods such as hotdogs (oil-in-water emulsion), food structure and lipid component may have a controlling influence on growth of the pathogen by their tendencies to redistribute chemical components between phases of foods and controlling the concentration of undissociated antimicrobial compounds in the aqueous phase (Brocklehurst et al., 1995; Brocklehurst and Wilson, 2000). As the undissociated form of organic acid is lipophilic, less of the undissociated acid or antimicrobial may localize in the aqueous phase and, hence, affect their efficacies (Von

schelhorn, 1964; Leo et al., 1971). Furthermore, between the chicken and turkey meat, variation in protein and fatty acid profiles exist (Komprda et al., 2002).

To our knowledge, there is no published literature on the influence of fat content on the growth of *L. monocytogenes* in presence of lactate and diacetate combinations in chicken and turkey hotdog formulations. Therefore, the main purpose of this study was to determine the effective combination of PL and SD to inhibit the growth of *L. monocytogenes* in surface inoculated low and high fat chicken and turkey hotdog model systems.

## **MATERIALS AND METHODS**

### **MATERIALS**

Mechanically separated chicken (24% fat) and fresh, boneless, skinless chicken breast (2.4% fat) (Tyson Foods Inc., Springdale, AR, USA), and mechanically deboned turkey with fat levels 7% and 24% (Cargill Meat Solutions, Springdale, AR, USA) were used in this study. Non-meat ingredients for hotdog preparation included salt, sodium tripolyphosphate, dextrose, monosodium glutamate, (Heartland Supp. Co, AR, USA), red pepper, black pepper (Eatam Foods Company, NJ, USA), sodium nitrite (Southern Indiana Butcher Supply, IN), potassium lactate (PURASAL<sup>®</sup> 60 % HiPure P, Purac America, Lincolnshire, IL), and sodium diacetate (Jarchem, NJ, USA). Non-edible casings were used to stuff the emulsified meat (Casings: 30 mm diameter fibrous cellulose casings; E-Z Peel4 Nojax, 30-84 4STR clear, Viskase Corp., Willowbrook, IL, USA). Modified oxford selective agar was used to isolate *Listeria monocytogenes* (EMD Chemicals Inc., Gibbstown, NJ, USA).

### **METHODS**

#### **Hotdog preparation**

High fat (20 % fat in final product) hotdogs were formulated using mechanically separated chicken or turkey meat; whereas the low fat (5 % fat in final product) hotdogs were

formulated using ground boneless, skinless chicken breast meat or mechanically separated turkey meat. Non-meat ingredients used in preparation of low and high fat chicken and turkey hotdogs included ice, salt, sodium tripolyphosphate, dextrose, sodium nitrite, dextrose, red and black pepper, and monosodium glutamate. Ground meat was mixed with non-meat ingredients and varying levels of PL and SD combinations (Fig. 3.1) to form a homogenous emulsion batter in a bowl chopper (Type K64V-VA, Seydelman, Germany). The meat emulsion was transferred to a sausage stuffer (Friedrich Dick hand stuffer, 15LTR, Germany) with inedible cellulose casings (30 mm diameter) and slid along the horn of the stuffer. This emulsion was stuffed, extruded, pinched, and twisted into 6-inch hotdogs links. Hotdogs were placed on cooking sticks in an oven (ALKAR-RapidPak, Inc., Model-1000, Wisconsin, USA) at 82.2 °C until the internal temperature reached 73.8 °C to kill/inactivate all the foodborne pathogens. After cooking, hotdogs were showered with water at 25.5 °C and stored at 4 °C for surface inoculation and storage studies.

### **Proximate analysis**

Determination of percent moisture (AOAC 2000, method 985.14), protein (AOAC 2000, method 992.15), and fat (AOAC 2000, method 985.15) were conducted in the hotdogs at the start of the experiment. Eight hotdogs (non-inoculated) per meat and fat type were homogenized in a food blender (Oster<sup>®</sup> 16-speed blender; Model-6687, Sunbeam Products, Inc., FL, USA) and sampled for moisture, protein, and fat analyses in triplicates.

### **Water activity ( $a_w$ )**

Homogenized hotdog samples were spread evenly up to half of the sample cup and positioned inside the vapour chamber of AquaLab<sup>™</sup> analyser (Model 3 series, Decagon Devices Inc., Washington DC, USA) at 20 °C to determine the  $a_w$  in triplicates.

## **Residual nitrite and pH**

Residual nitrite (ppm) in hotdogs (meat and fat type) was determined in triplicate samples by colorimetric method (AOAC 2000, method 973.31). For pH determination, frankfurter samples were first stomached for 120 s at 8.0 strokes/s (Neutec Group Inc.191 masticator; Torrent de l'Estadella, 22 08030 Barcelona, Spain) with distilled water (1:10 w/v). The pH values were recorded by using a pH meter (Orion™, model 720A, Orion Research Inc., Beverly, MA, USA) into the stirred slurry.

## **Preparation of bacterial suspension**

A loopful of frozen stock (at -70 °C) of *Listeria monocytogenes* (strain V7, serotype ½ a; FDA isolate) obtained from Center for Food Safety Laboratory (Fayetteville, AR) was transferred to brain heart infusion (BHI) broth (10 mL) and incubated (New Brunswick Scientific agitating incubator at 200 rpm; Edison, NJ, USA) at 37 °C for 24 h. About 10 µL of the culture was inoculated into 10 mL of fresh BHI, and incubated at 37 °C for 18 h. The incubated cultures after 18 h were centrifuged (J2–21 Centrifuge, Beckman, Fullerton, CA, USA) at 25 °C for 10 min to obtain the supernatant and the culture pellets. The pellets were washed twice with phosphate buffer saline (PBS) and resuspended in the volume of PBS that was equal to the original volume of BHI in the culture. Serial dilutions of the bacterial suspension were made to obtain approximately 10<sup>5</sup> cfu/mL for surface inoculation of the hotdog samples.

## **Surface inoculation of the hotdog samples**

Hotdogs were sliced into cubes (1.5 - 2 g; 1 cm<sup>3</sup>) and used for surface inoculation as described by Gadang et al. (2008). We used cubed hotdog samples (for better handling and control over experimental conditions under a laboratory setting) as a model system instead of full hotdogs for surface inoculation as demonstrated by previous studies (Lungu et al., 2005 a, b;

Theivendran et al., 2006; Sivarooban et al., 2007). For surface inoculation, hotdog samples were dipped into the *L. monocytogenes* cultures ( $\sim 10^5$  cfu/mL) for 1 min and air dried for 20 min under a laminar hood that enabled the bacterial cells to attach to the surface of hotdog samples (to achieve  $\sim 4.6$  log cfu/g). A total of 588 hotdog samples [2 meat types (chicken and turkey) X 2 fat levels (low and high) X 7 treatments including control X 7 sampling days X 3 replications] were inoculated. After drying, each inoculated hotdog sample was transferred to a sterile whirlpak bag (7.5 x 12.5 cm; 2.25 mil thickness and 0.178CC/100 Sq m), vacuum packed (VacMaster-VP 215, Portland, OR, USA), and stored at 4 °C for 28 days.

### **Bacterial enumeration**

Three hotdog samples per treatment were observed on each sampling day 4, 8, 12, 16, 21, and 28 days to determine the inhibitory activity of PL and SD combination against the growth of *L. monocytogenes*. Phosphate buffer saline (PBS at pH 7.0) was added to the stomacher bags to make a 10 fold dilution and stomached for 120s to form a homogenate. Stomached samples were serially diluted with PBS, spread plate on oxford agar with selective supplement, and incubated at 37 °C for 48 h for colony enumeration.

### **Experimental design and statistical analysis**

The experiment design was a split plot where the whole plot portion was completely randomized with [two meats (chicken and turkey) X two fats (high and low fat) X seven PL-SD combinations (including control i.e. no PL and SD)] and the split-plot factor was the six storage times (4, 8, 12, 16, 21, and 28 days) with three replications (3 hot dog samples at each sampling time). Analysis of variance was performed using PROC MIXED in SAS<sup>®</sup> version 9.2 (SAS Institute, Cary, NC, USA) and used to determine statistical differences among the main effects and their interactions with a significance level of  $P < 0.05$ . Significant differences among the

least squares growth means were used ( $P < 0.05$ ) among the treatments to identify the effective PL-SD combination. For each storage time (day), growth inhibitions (log cfu/g) were determined as the difference between mean log (cfu/g) count of the control and the mean log (cfu/g) count of treatment sample (PL-SD combination) on that particular day.

## RESULTS AND DISCUSSION

### **Proximate analyses, pH and water activity of hotdog samples:**

The percent moisture, fat, protein, residual nitrite, and pH of the hotdog samples, determined on day zero, are presented in Table 3.1 (A & B). Protein content was significantly higher ( $P < 0.05$ ) in low fat chicken hotdogs when compared to the other hotdog samples (high fat chicken, low and high fat turkey hotdogs), as it was prepared from skinless, boneless chicken breast. Furthermore, there were significant differences ( $P < 0.05$ ) between chicken and turkey hotdogs in residual nitrite content (ppm) with chicken hotdog samples having significantly higher ( $P < 0.05$ ) nitrite than turkey samples (Table 3.1A).

The initial pH of the control samples (no lactates and diacetates) was 6.12 to 6.50 in low and high fat chicken and turkey hotdogs (Table 3.1B). Addition of antimicrobials at varying combination levels significantly ( $P < 0.05$ ) reduced the pH. Hotdog samples formulated with higher levels of PL (2.0 % or 3.0 %) and SD (0.15 % or 0.2 %) reduced the pH in all meat and fat treatments (Table 3.1B). Addition of PL and SD at varying levels in hotdog formulations did not significantly ( $P > 0.05$ ) reduce the water activity ( $a_w$ ) (data not shown) when compared to control samples suggesting that these samples provide favourable conditions for the growth of *L. monocytogenes* (Lado and Yousef, 2007).

### **Effect of PL and SD combinations against the growth of *Listeria monocytogenes*:**

#### ***In low and high fat chicken hotdog samples***

Growth of *L. monocytogenes* on surface inoculated low and high fat chicken hotdog samples are presented in figure 3.2 (A & B). In low and high fat chicken hotdogs, control samples that did not have PL and SD combination supported rapid growth of *L. monocytogenes* until spoilage ( $\geq 9.0$  log cfu/g by 28 days of storage). However, incorporation of PL and SD at various combination levels achieved variable levels of growth inhibition of *L. monocytogenes* (Fig 3.2 A & B). Treatments with 1.0% PL and 0.15% SD and 1.0% PL and 0.20% did not effectively inhibit the growth of *L. monocytogenes* due to low concentration of antimicrobials (lactates and diacetates) in the hotdog samples. Hotdogs formulated with higher levels of PL-SD combinations (2 – 3 % PL and 0.15 – 0.20 % SD) demonstrated more growth inhibition compared to control. In chicken control samples, growth of *L. monocytogenes* was higher in high fat samples than low fat samples on all observation days. Considering the growth of *L. monocytogenes*, there were no significant differences ( $P > 0.05$ ) between low and high fat samples until 4<sup>th</sup> day of storage (4 °C). However, significant differences ( $P < 0.05$ ) in growth between low and high fat samples were apparent from 8<sup>th</sup> day with higher growth inhibition values (log cfu/g) in low fat samples. Considering the growth inhibitions, the most effective treatment in low and high fat chicken hotdog samples was the combination of 3.0 % PL and 0.15 % SD with 3.42 log cfu/g and 2.36 log cfu/g growth inhibitions on the 16<sup>th</sup> and 21<sup>st</sup> days of storage respectively. Higher growth inhibitions in low fat hotdog samples can be attributed to increased action of these antimicrobials in the water phase, thus exhibiting the inhibitory activities against the growth of *L. monocytogenes*. These results were not consistent with findings of Hu and Shelef (1996) that inhibitory activities of lactates increased with fat content in the beaker sausages. Furthermore, previous studies conducted to determine the effect of fat content on the growth behaviour of *L. monocytogenes* in dairy foods such as cheese and yogurt

reported that fat content had no effect on the growth pattern of *L. monocytogenes* (Griffith and Deibel, 1990; Mehta and Tatini, 1994). These differences may be due to the variation in the sensitivity of the strain used in dairy (Scott A vs V 7), product characteristics (pH, moisture, water activity), and model system matrix.

### ***In low and high fat turkey hotdog samples***

Growth of *L. monocytogenes* in low and high fat turkey control hotdog samples at over 28 days of storage at 4 °C is presented in figure 3.3 (A & B). In low and high fat turkey samples, maximum growth observed by 28 days of storage at 4 °C was 8.05 log cfu/g and 6.24 log cfu/g respectively. Interestingly, growth of *L. monocytogenes* in turkey high fat samples was low over 28 days of storage at 4 °C. For example, in high fat control samples, *L. monocytogenes* population grew from 4.68 log cfu/g to 6.42 log cfu/g by 28 days. Furthermore, in turkey high fat samples, treatments formulated with 2.0 % PL and 0.15 % SD or higher [(T; PL %, SD %): (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)] demonstrated listeristatic activity until 16 days of storage. However, this growth behaviour in high fat system was not consistent in the low fat samples. Considering the growth of *L. monocytogenes*, there were significant differences ( $P < 0.05$ ) between low and high fat samples on all sampling days of storage. Growth inhibitions were higher in low fat turkey samples until 21<sup>st</sup> day and thereafter, while high fat samples exhibited higher growth inhibitions on 28<sup>th</sup> day. The most effective treatment in low and high fat turkey hotdog samples was the combination of 3.0 % PL and 0.20 % SD with 3.18 log cfu/g and 1.60 log cfu/g growth inhibition on 16<sup>th</sup> and 12<sup>th</sup> day of storage respectively (Fig. 3.3 A & B).

Pathogen survivors following antimicrobial intervention (stressor: lactate and diacetate combination) stimulate their protective mechanisms (Ricke et al., 2005). However, inclusion of additional hurdles (storage temperature and vacuum packaging) that contribute to different

mechanism(s) may be more effective in pathogen inhibition (Ricke et al., 2005). Inhibition of *L. monocytogenes* in this study would be due to several factors. A combination of factors such as lactate and diacetate combinations, pH, vacuum packaging (reduce the redox-potential), and storage temperature may have resulted in the varying antimicrobial activity of the treatments, suggesting that a multiple hurdle approach can inhibit the growth of *L. monocytogenes* during contamination (Chen & Shelef, 1992; Buchanan et al., 1993). Potassium lactate and sodium diacetate are widely used in combination in various meat and poultry products to enhance food safety and extend shelf life (Kalinowski and Tompkin 1999; Nerbrink et al., 1999; Porto and Franco, 2002; Stekelenburg, 2003; Nunez et al., 2004; Knight and Castillo, 2007). Findings as discussed in previous sections were consistent with previous research findings that demonstrated varying levels of inhibition with PL-SD combination levels between 2 to 3% PL and 0.15 to 0.2% SD in various meat models (Stekelenburg and Kant-Muermans, 2001; Mbandi and Shelef, 2002; Stekelenburg, 2003).

These results also suggested that fat level has significant effect on the antimicrobial activity of the lactates and diacetates against the growth of *L. monocytogenes*. It is likely that high fat samples with lower growth inhibition could be due to the protective influence of fat (less water, pathogen cell protection) from the interaction of lactates and diacetates with the pathogen. This protective effect could either be due to physical protection of the bacterial cell or due to other types of interaction between fat in the meat and bacterial cell wall lipids (Mehta and Tatini, 1994). In addition, in bi-phasic foods such as hotdogs (oil-in-water emulsion), the lipid component of the food is vital in controlling the concentration of undissociated antimicrobial compounds in the aqueous phase. This is due to the lipophilic nature of the undissociated nature of the organic acids, partition between aqueous and lipid components of foods, and thus,

ultimately, decrease the concentration of undissociated organic acids (lactates and diacetates) in the aqueous phase (Leo et al., 1971; Brocklehurst and Wilson, 2000).

Differences in the effective combinations of PL-SD determined in this study between meat and fat types against *L. monocytogenes* can also be attributed to the chemical composition and food structure, and, thus, may affect the bacterial attachment and growth of the pathogen (Brocklehurst and Wilson, 2000; Barmpalia et al., 2005). Composition of meat, type, and level of unsaturated fatty acids present in chicken and turkey meat, and based on low (5%) and high (20%) fat hotdogs, all may contribute to variances, as the unsaturated fatty acids are known to inhibit gram-positive foodborne pathogens such as *L. monocytogenes* (Nieman, 1954; Kabara, 1978; Mbandi et al., 2004). When compared to chicken, turkey meat has higher amounts of unsaturated fatty acids such as linoleic (PUFA) that have inhibitory activities against *L. monocytogenes* (Ratnayake et al., 1989; Komprda et al., 2002). Certain nutrients such as albumins (lipophilic proteins), globulins, fat globules, starches, and others could interact with antimicrobial components that decrease their availability (Kabara, 1978). To further strengthen this conclusion, Wang and Johnson (1992) reported that inhibition of *L. monocytogenes* by unsaturated fatty acids in milk is dependent on the fat content in addition to temperature and pH.

## CONCLUSIONS

Combination of 3.0 % PL and 0.15 % or 0.20 % SD demonstrated effective growth inhibitions (approximately 3.0 log cfu/g) against *L. monocytogenes* than the treatments formulated with lower levels of PL and SD. High fat (chicken or turkey) demonstrated lower growth inhibitions of *L. monocytogenes* than low fat samples. Current usage levels of PL ( $\leq 2\%$ ) and diacetates combination in commercial hotdog formulations may not provide effective inhibition of *L. monocytogenes* when the product becomes contaminated. Therefore, other hurdle

technologies in addition to using effective concentration levels of chemical antimicrobials need investigation to obtain minimum detectable levels of pathogens.

## REFERENCES

- Barmpalia, I.M., Koutsoumanis, K.P., Geornaras, I., Belk, K.E., Scanga, J.A., Kendall, P.A., Smith, G.C. and Sofos, J.N. 2005. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10°C. *Food Microbiol*, 22, 205-211.
- Blom, H., Nerbrink, E., Dainty, R., Hagtvedt, T., Borch, E., Nissen, H. and Nesbakken, T. 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum-packed, sensory-acceptable serelat sausage and cooked ham stored at 4 °C. *Int J Food Microbiol*, 38, 71–76.
- Brewer, S., Keith, F., Martin, S., Dallmier, A., and Meyer, J. 1991. Sodium lactate effects on shelf-life sensory and physical characteristics of fresh pork sausage. *J Food Sci*, 56, 1176–1178.
- Brocklehurst, T.F., Parker, M.L., Gunning, P.A., Coleman, H.P. and Robins, M.M. 1995. Growth of foodborne pathogenic bacteria in oil-in-water emulsions: II Effect of emulsion structure on growth parameters and form of growth. *J Appl Bacteriol*, 78, 609-615.
- Brocklehurst, T.F. and Wilson, P.D.G. 2000. The role of lipids in controlling microbial growth. *Grasasy Aceites*, 51, Fasc. 1-2, 66-73.
- Buchanan, R.L., Golden, M.H. and Whiting, R.C. 1993. Differentiation of the effects of pH and lactic or acetic acid concentration on the kinetics of *Listeria monocytogenes* inactivation. *J Food Prot*, 56, 474–478.
- CDC. 2002. Public health dispatch: outbreak of listeriosis—Northeastern United States, 2002. *MMWR*, 51, 950–951.
- Chen, N. and Shelef, L.A. 1992. Relationship between water activity, salts of lactic acid, and growth of *Listeria monocytogenes* in a meat model system. *J Food Prot*, 55, 574–578.
- Corbo, M.R., Bevilacqua, A., Campaniello, D., D'amato, D., Speranza, B. and Sinigaglia, M. 2009. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches – a review. *Int J Food Sci Technol*, 44, 223–241.
- Debevere, J.M. 1989. The effect of sodium lactate on the shelf-life of vacuum-packed coarse liver pâté. *Fleischwirtsch*, 69, 223–224.
- FDA. 2000. Food additives for use in meat and poultry products: sodium diacetate, sodium acetate, sodium lactate and potassium lactate. *Federal Register*, 65, 3121–3123.
- FDA. 2001. Performance standards for the production of processed meat and poultry products: proposed rule. *Federal Register*, 66, 12589–12636.

- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *J Food Sci.*, 73, 389-394.
- Glass, K.A. and Doyle, M.P. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl Environ Microbiol*, 55, 1565– 1569.
- Gooding, C.M., Melnick, D., Lawrence, R.L. And Luckmann, F.H. 1955. Sorbic acid as a fungistatic agent for foods. IX. Physicochemical considerations in using sorbic acid to protect foods. *Food Res*, 20, 639-648.
- Gottlieb, S.L., Newbern, E.C., Griffin, P.M., Graves, L.M., Hoekstra, R.M., Baker, N.L., Hunter, S.B., Holt, K.G., Ramsey, F., Head, M., Levine, P., Johnson, G., Schoonmaker-Bopp, D., Reddy, V., Kornstein, L., Gerwel, M., Nsubuga, J., Edwards, L., Stonecipher, S., Hurd, S., Austin, D., Jefferson, M.A., Young, S.D., Hise, K., Chernak, E.D. and Sobel J. 2006. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in U.S. regulatory policy. *Clin. Infect Dis*, 42, 29–36.
- Griffith, M. and Decibel, K.E. 1990. Survival of *Listeria monocytogenes* in yogurt with varying levels of fat and solids. *J. Food Saf*, 10, 219-230.
- Houtsma, P.C., Dewit, J.C. and Rombouts, F.M. 1996a. Minimum inhibitory concentration (MIC) of sodium lactate and sodium chloride for spoilage organisms and pathogens at different pH values and temperatures. *J Food Protect*, 59, 1300–1304.
- Houtsma, P.C., Kant-Muermans, M.L., Rombouts, F.M. and Zwietering, M.H. 1996b. Model for the combined effects of temperature, pH, and sodium lactate on growth rates of *Listeria innocua* in broth and bologna-type sausages. *Appl Environ Microbiol*, 62, 1616–1622.
- Hu, A.C. and Shelef, L.A. 1996. Influence of fat content and preservatives on the behavior of *Listeria monocytogenes* in beaker sausage. *J. Food Saf*, 16, 175–181.
- Hunter, D.R. and Segal, I.H. 1973. Effect of weak acids on amino acid transport by *Penicillium chrysogenum*: evidence for proton or charge gradient as the driving force. *J Bacteriol*, 113, 1184–1192.
- Kabara, J.J. 1978. Fatty acids and derivatives as antimicrobial agents. A review, p. 1-14. In J.J. Kabara (ed.), *The pharmacological effect of lipids*, vol. I. AOCS, Champaign, IL.
- Kalinowski, R.M., and Tompkin, R.B. 1999. Psychrotropic *Clostridia* causing spoilage in cooked meat and poultry products. *J Food Prot*, 62, 766–772.
- Keeton, J.T. 2001. Formed and emulsion products. In *Poultry Meat Processing* (edited by A.R. Sams) pp. 195–226. CRC Press, Boca Raton, FL.

- Klontz, K.C., McCarthy, P.V., Datta, A.R., Lee, J.O., Acheson, D.W.K. and Brackett, R.E. 2008. Role of the US Food and Drug Administration in the regulatory management of human listeriosis in the United States. *J Food Prot*, 71, 1277–1286.
- Knight, T.D. and Castillo, A. 2007. Effectiveness of Potassium lactate and sodium diacetate in combination with irradiation to control *Listeria monocytogenes* on frankfurters. *J Food Sci*, 72, 26-30.
- Komprda, T., Sarmanova, I., Zelenka, J., Bakaj, P., Fialova, M. and Fajmonova, E. 2002. Effect of sex and age on cholesterol and fatty acid content in turkey meat. *Arch Geflugelk*, 66, 263-273.
- Lado, B.H. and Yousef, A.E. 2007. Characteristics of *Listeria monocytogenes* important to food processors, p. 157-213. In E.T. Ryser and E.H. Marth (eds.), *Listeria*, listeriosis and food safety, 3rd ed. Marcel Dekker, Inc., New York, NY.
- Leo, A., Hansch, C. and Elkins, D. 1971. Partition coefficients and their uses. *Chem Rev*, 71, 5-631.
- Lianou, A. and Sofos, J.N. 2007. A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. *J Food Prot*, 70, 2172–2198.
- Lungu, B. and Johnson, M.G. 2005a. Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4 °C. *J Food Prot*, 68, 855–859.
- Lungu, B. and Johnson, M.G. 2005b. Potassium sorbate does not increase control of *Listeria monocytogenes* when added to zein coatings with nisin on the surface of full fat turkey frankfurter pieces in a model system at 4°C. *J Food Sci*, 70, 95–99.
- Maas, M.R, Glass, K.A. and Doyle, M.P. 1989. Sodium lactate delays toxin production by *Clostridium botulinum* in cook-in-bag turkey products. *Appl Environ Microbiol*, 55, 2226-2229.
- Mbandi, E., and Shelef, L.A. 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. *J Food Prot*, 64, 640–644.
- Mbandi, E., and Shelef, L.A. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int J Food Microbiol*, 76, 191-198.
- Mbandi, E., Brywig, M., and Shelef, L.A. 2004. Antilisterial effects of free fatty acids and monolaurin in beef emulsions and hot dogs. *Food Microbiol*, 21, 815–818.

- Mead, P.S., Slutsker, L., Dietz, V., Mccaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M. and Tauxe, R.V. 1999. Food-related illness and death in the United States. *Emerg Infect Diseases*, 5, 607–625.
- Mehta, A. and Tahini, S.R. 1994. An evaluation of the microbiological safety of reduced-fat cheddar-like cheese. *J Food Prot*, 57, 776-779.
- Miller, R.K. and Acuff, G.R. 1994. Sodium lactate affects pathogens in cooked beef. *J Food Sci*, 59, 15-19.
- Nerbrink, E., Borch, E., Blom, H. and Nesbakken, T. 1999. A model based on absorbance data on the growth of *Listeria monocytogenes* and including the effects pH, NaCl, sodium lactate and sodium acetate. *Int J Food Microbiol*, 47, 99–109.
- Nieman, C. 1954. Influence of fatty acids on the growth of microorganisms. *Bact Rev*, 18, 147-163.
- Nuñez D.G, Keeton, J.T. and Ringer, L.J. 2004. Effectiveness of acidic calcium sulfate with propionic and lactic acid and lactates as post processing dipping solutions to control *Listeria monocytogenes* on frankfurters with or without potassium lactate and stored vacuum packaged at 4.5 C. *J Food Prot*, 67, 915-921.
- Olsen, S.J., Patrick, M., Hunter, S.B., Reddy, V., Kornstein, L., Mackenzie, W.R., Lane, K., Bidol, S., Stoltman, G.A., Frye, D.M., Lee, I., Hurd, S., Jones, T.F., Laporte, T.N., Dewitt, W., Graves, L., Wiedmann, M., Schoonmaker-Bopp, D.J., Huang, A.J., Vincent, C., Bugenhagen, A., Corby, J., Carloni, E.R., Holcomb, M.E., Woron, R.F., Zansky, S.M., Dowdle, G., Smith, F., Ahrabi-Fard, S., Ong, A.R., Tucker, N., Hynes, N.A. And Mead, P. 2005. Multistate outbreak of *Listeria monocytogenes* infection linked to delicatessen turkey meat. *Clin Infect Dis*, 40, 962–967.
- Porto, A.C.S. and Franco, B.D.G.M. 2002. Viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters, commercially prepared with and without 2.0 or 3.0% added potassium lactate, during extended storage at 4 and 10 °C. *J Food Prot*, 65, 308- 315.
- Ratnayake, W.M.N., Ackman, R.G., and Hulan, H.W. 1989. Effect of redfish meal enriched diets on the taste and N-3 PUFA of 42-day-old broiler chickens. *J Sci Food Agric*, 49, 59-74.
- Ricke, S.C., Kundinger, M.M., Miller, D.R. and Keeton, J.T. 2005. Alternatives to antibiotics: Chemical and physical antimicrobial interventions and foodborne pathogen response. *Poult Sci*, 84, 667–675.
- Rocourt, J., Benembarek, P., Toyofuku, H. and Schlundt, J. 2003. Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: the FAO/WHO approach. *FEMS Immunol Med Microbiol*, 35, 263–267.

- Salmond, C.V., Kroll, R.G. and Booth, I.R. 1984. The effects of food preservatives on pH homeostasis in *Escherichia coli*. *J Gen Microbiol*, 130, 2845–2850.
- Seman, J.L., Borger, A.C., Meyer, J.D., Hall, P.A. And Milkowski, A.L. 2002. Modeling of the growth of *Listeria monocytogenes* in cured ready-to-eat meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. *J Food Prot*, 65, 651–658.
- Shelef, L.A. and Addala, L. 1994. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. *J. Food Saf*, 14, 103-115.
- Sivarooban, T., Hettiarachchy, N. S. and Johnson, M.G. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10°C. *J Food Prot*, 70, 1017–1020.
- Sommers, C.H., Fan, X., Niemira, B.A. and Sokorai, K. 2003. Radiation (gamma) resistance and post-irradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. *J. Food Prot*, 66, 2051–2056.
- Stekelenburg, F.K. and Kant-Muermans, M.L.T. 2001. Effects of sodium lactate and other additives in a cooked ham product on sensory quality and development of a strain of *Lactobacillus curvatus* and *Listeria monocytogenes*. *Int J Food Microbiol*, 66, 197–203.
- Stekelenburg, F.K. 2003. Enhanced inhibition of *Listeria monocytogenes* in frankfurter sausage by the addition of potassium lactate and sodium diacetate mixtures. *Food Microbiol*, 20, 133–137.
- Theivendran, S., Hettiarachchy, N.S., and Johnson, M.G. 2006. Inhibition of *Listeria monocytogenes* by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *J Food Sci*, 71, 39–44.
- Von Schelhorn, M. 1964. Distribution of preservatives between fat and water in foods. In *Microbial inhibitors in food. Proceedings of the Fourth International Symposium on Food Microbiology*, Göteborg, Sweden. Stockholm, Göteborg, Uppsala, Almquist & Wiksell.
- Wang, L.L. and Johnson, E.A. 1992. Inhibition of *Listeria monocytogenes* by fatty acids and monoglycerides. *Appl Environ Microbiol*, 58, 624-629.
- Weber, A.J. 1997. Palatability of roast beef and turkey injected with salts of various organic acids. M.S. thesis, Texas A&M University, College Station, TX.

**Table 3.1A.** Percent moisture, protein, fat, and residual nitrite determined in low and high fat chicken and turkey hotdogs (No lactate and diacetate).

Treatment		Moisture (%)	Protein (%)	Fat (%)	Residual nitrite (ppm)
Meat	Fat				
Chicken	Low	71.26 <sup>a</sup>	20.12 <sup>a</sup>	5.12 <sup>b</sup>	0.99 <sup>a</sup>
	High	60.67 <sup>b</sup>	15.91 <sup>b</sup>	20.51 <sup>a</sup>	1.17 <sup>a</sup>
Turkey	Low	69.24 <sup>a</sup>	15.56 <sup>b</sup>	5.08 <sup>b</sup>	0.59 <sup>b</sup>
	High	58.24 <sup>b</sup>	14.57 <sup>b</sup>	20.34 <sup>a</sup>	0.47 <sup>b</sup>

Values are the average of three replications.

Means followed by same superscripts in the same column are not significantly different ( $P > 0.05$ ).

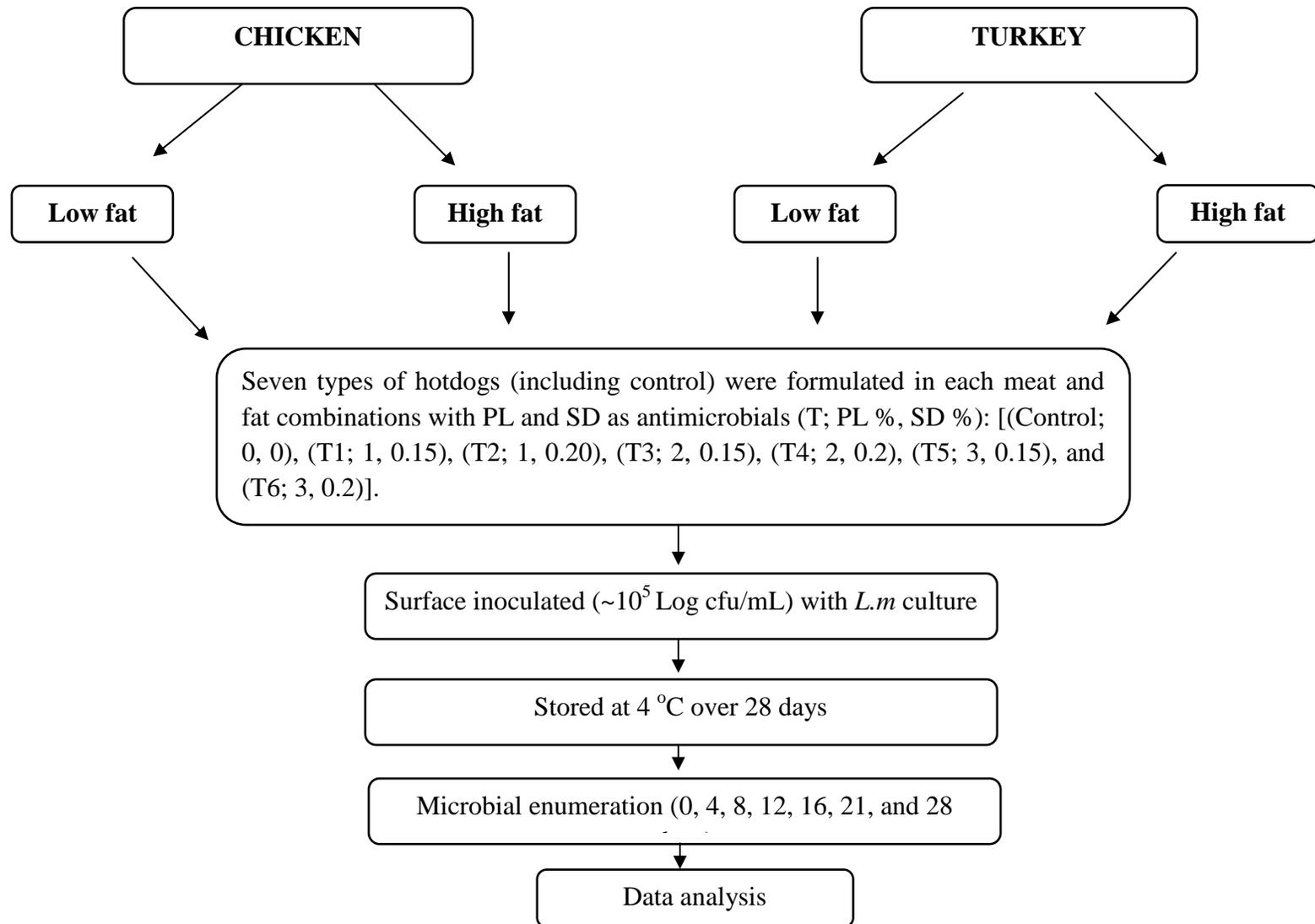
**Table 3.1B.** pH values determined in low and high fat chicken and turkey hotdog samples.

Treatment	PL %	SD%	Chicken		Turkey	
			Low fat	High fat	Low fat	High fat
<b>C</b>	0.00	0.00	6.12 <sup>a</sup>	6.25 <sup>a</sup>	6.50 <sup>a</sup>	6.40 <sup>a</sup>
<b>T1</b>	1.00	0.15	6.09 <sup>b</sup>	6.21 <sup>a</sup>	6.45 <sup>a</sup>	6.36 <sup>a</sup>
<b>T2</b>	1.00	0.20	6.05 <sup>b</sup>	6.00 <sup>b</sup>	6.30 <sup>c</sup>	6.25 <sup>bc</sup>
<b>T3</b>	2.00	0.15	6.03 <sup>c</sup>	6.03 <sup>b</sup>	6.36 <sup>b</sup>	6.20 <sup>c</sup>
<b>T4</b>	2.00	0.20	5.94 <sup>d</sup>	5.93 <sup>c</sup>	6.26 <sup>d</sup>	6.12 <sup>d</sup>
<b>T5</b>	3.00	0.15	6.01 <sup>c</sup>	6.02 <sup>b</sup>	6.27 <sup>cd</sup>	6.26 <sup>b</sup>
<b>T6</b>	3.00	0.20	5.94 <sup>d</sup>	5.93 <sup>c</sup>	6.20 <sup>e</sup>	6.15 <sup>d</sup>

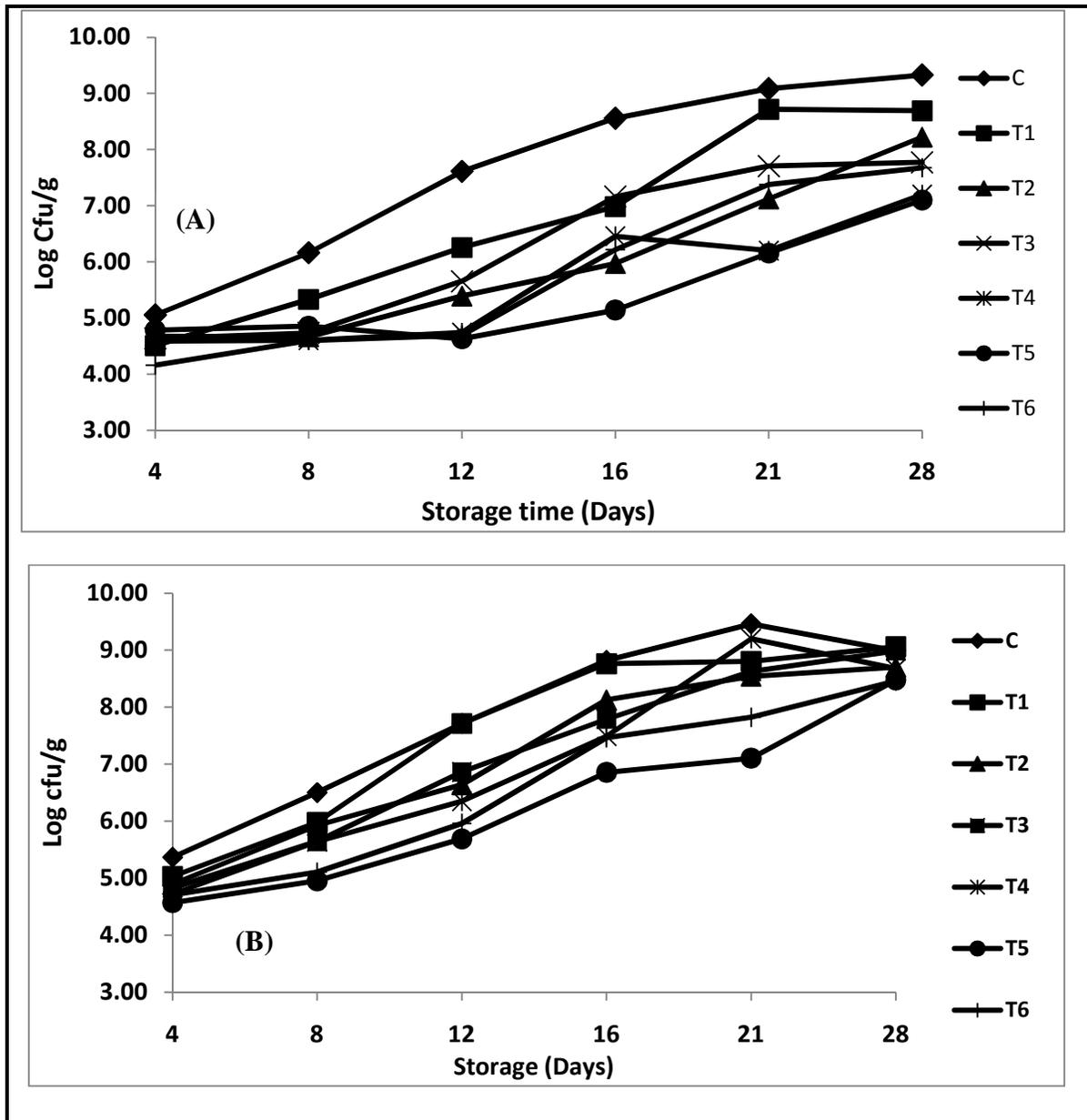
Values are the average of three replications.

Means followed by same superscripts in the same column are not significantly different ( $P > 0.05$ ).

Treatments - (T; PL, SD): [(Control; 0, 0), (T1; 1.0, 0.10), (T2; 1.0, 0.20), (T3; 2.0, 0.15), (T4; 2.0, 0.20), (T5; 3.0, 0.15) and (T6; 3.0, 0.20)]



**Figure 3.1** Schematic diagram of experimental design involving low and high fat chicken and turkey hotdog treatments to determine the effective combination of potassium lactate and sodium diacetate against surface inoculated *Listeria monocytogenes*.



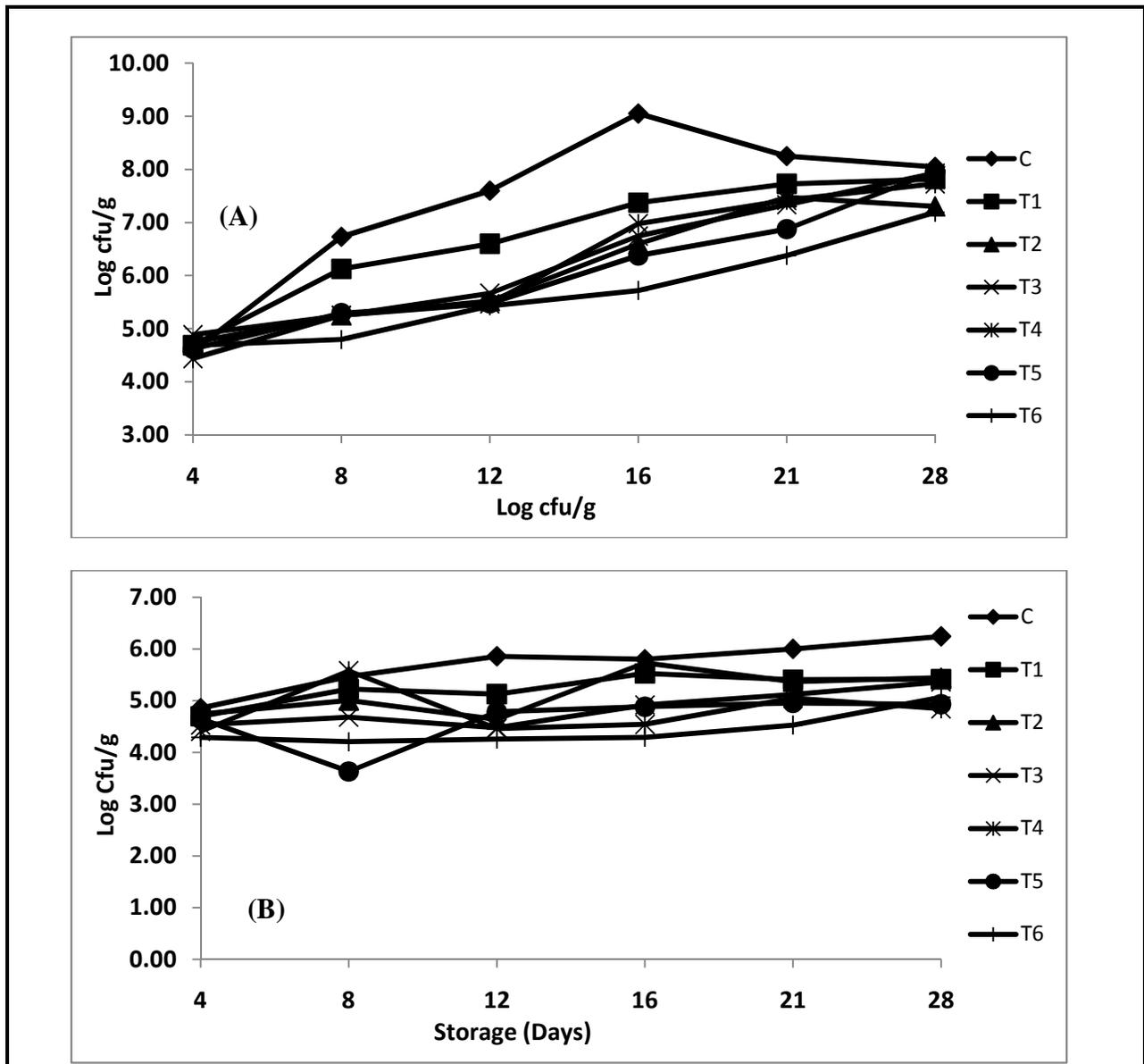
**Figure 3.2 (A & B).** Effect of potassium lactate (PL) and sodium diacetate (SD) combination against *Listeria monocytogenes* in (A) low fat and (B) high fat chicken hotdogs vacuum packed and stored at 4 °C.

Data are mean log numbers (cfu/g) from three replications observed on 4, 8, 12, 16, 21, and 28 days of storage.

Treatments - (T; PL %, SD %): [(Control; 0, 0), (T1; 1, 0.15), (T2; 1, 0.2), (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)]. Minimum level of detection is 100 cfu/g.

LSD to compare means to determine significant differences ( $P < 0.05$ ) at within same meat or fat or PL-SD combinations = 0.19

LSD to compare means to determine significant differences ( $P < 0.05$ ) at different meat or fat or PL-SD combinations = 0.25



**Figure 3.3 (A & B).** Effect of potassium lactate (PL) and sodium diacetate (SD) combination against *Listeria monocytogenes* in (A) low fat and (B) high fat turkey hotdogs vacuum packed and stored at 4 °C.

Data are mean log numbers (cfu/g) from three replications observed on 4, 8, 12, 16, 21, and 28 days of storage.

Treatments - (T; PL%, SD %): [(Control; 0, 0), (T1; 1, 0.15), (T2; 1, 0.2), (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)]. Minimum level of detection is 100 cfu/g.

LSD to compare means to determine significant differences ( $P < 0.05$ ) at within same meat or fat or PL-SD combinations = 0.19

LSD to compare means to determine significant differences ( $P < 0.05$ ) at different meat or fat or PL-SD combinations = 0.25

## CHAPTER IV

### EFFECT OF REDUCING POTASSIUM LACTATE, SODIUM DIACETATE, AND INCORPORATING PLANT EXTRACTS, NISIN, AND EDTA COMBINATIONS ON INHIBITING *LISTERIA MONOCYTOGENES* INOCULATED IN HOTDOG MODEL SYSTEM

#### ABSTRACT

The main objective of this study was to investigate the effect of reducing potassium lactate (PL) and sodium diacetate (SD) levels and incorporating green tea extract (GTE), grape seed extracts (GSE), nisin, and EDTA combinations to inhibit *L.m.* in ready-to-eat high (20 %) and low fat (5 %) chicken and turkey hotdogs. Optimal levels of PL (3.0 %), SD (0.15 %), GTE (1.4 %), and GSE (0.9 %) were used in this study. Seven treatments [**T1**: Control (no chemicals or plant extracts); **T2**: PL-SD; **T3**: GTE-GSE; **T4**: 25 % (PL-SD) + 75 % (GSE-GTE); **T5**: 50 % (PL-SD) + 50 % (GSE-GTE); **T6**: 75 % (PL-SD) + 25 % (GSE-GTE); **T7**: PL-SD (commercial level: 2.0 % and 0.15 % respectively)] were used to determine the effective combination of chemical antimicrobials and plant extracts that would inhibit the growth of *L.m.* Hotdog samples (low and high fat chicken and turkey) formulated with plant extracts alone demonstrated growth inhibitions against *L.m.* until the 8<sup>th</sup> day storage, and thereafter grew to 8.3 log cfu/g (spoiled by 28 days). No significant differences ( $P > 0.05$ ) in growth inhibitions were observed between treatments formulated with commercial level of PL and SD (T7) and treatments formulated with chemical preservatives and plant extracts (T6). Low fat hotdogs (chicken and turkey) formulated with chemical preservatives and plant extracts have demonstrated significantly higher ( $P < 0.05$ ) growth inhibitions of *L.m.* compared to high fat hotdogs. This study suggested that chemical preservatives used in hotdogs can be partially replaced (25 % level) by natural plant extracts and can be more effective when used with other multiple hurdle technology combinations such as thermal treatments, electrostatic spraying, and nanotechnology.

## INTRODUCTION

Despite several efforts in reducing the contamination of food products, foodborne illness implicated by *Listeria monocytogenes* is still a major concern to processors and consumers (USDA/ERS, 2010). In 2009, the number of reported infections and incidence (per 100,000 populations) by *Listeria* was 0.34 % (CDC, 2010). There has been a continued interest focused by researchers and processors to explore natural alternatives and effective strategies to control the foodborne pathogen contamination (Sivarooban et al., 2007; Juneja et al., 2010; Xia et al., 2010). Plant extracts having phenolic-rich compounds that are derived from sage, rosemary, thyme, hops, green tea extract, grape seed extract, cloves, and basil are known to have antimicrobial effects against foodborne pathogens such as *L. monocytogenes*, *S. Typhimurium*, and *Campylobacter* (Davidson and Naidu, 2000; Elgayyar et al., 2001; Sivarooban et al., 2007; Over et al., 2009; Perumalla and Hettiarachchy, 2010; Ganesh et al., 2011). Several studies have investigated the effect of plant extracts alone or in combination with chemical preservatives in limiting the growth of foodborne pathogens (Juneja et al., 2010; Xia et al., 2011). Natural plant extracts along with other hurdles such as low level of chemical preservatives, post-process thermal treatments, and irradiation demonstrated enhanced antimicrobial activities in meat and poultry products (Beuchat et al., 1994; Juneja et al., 2010; Over et al., 2010)

Purified catechin fractions from green tea have shown antimicrobial properties in broth as well as meat model systems (Yilmaz, 2006; Banon et al., 2007; Over et al., 2009). Catechins have a deteriorating effect on the lipid bilayer membrane that results in loss of cell structure and function and finally leads to the cell death (Tsuchiya et al., 1996 and Cox et al., 2001). Jayaprakasha et al. (2003) demonstrated the antimicrobial properties of GSE against *Bacillus*, *Staphylococcus*, *Pseudomonas aeruginosa*, and *E. coli*. Grape seed extracts have also

demonstrated antimicrobial activities against *L. monocytogenes*, *E. coli* O157:H7, and *S. Typhimurium* in meat products (Ahn et al., 2007; Gadang et al., 2008). The principal antibacterial compound found in methanol extract from grape seeds is gallic acid that had inhibitory effects on *E. coli* and *S. enteridis* (Shoko et al., 1999).

Nisin is a 34 amino acid bacteriocin (antimicrobial peptides produced by bacteria) produced by *Lactococcus lactis* strains during its exponential phase of growth (Davidson and Harrison, 2002) and is approved (GRAS) in the U.S. for use under 21 CFR 184.1538 and over 50 countries in food preservation. Though nisin is effective against spores and gram-positive bacteria such as *L. monocytogenes*, it does not have a significant inhibitory effect on gram-negative organisms, yeasts or moulds (Boziaris and Adams, 1999; Cannarsi et al., 2008). However, it can be effectively used when combined with chelating agents such as ethylenediaminetetraacetic acid (EDTA) that make the cell walls permeable to nisin (Boziaris and Adams, 1999). Previous studies have demonstrated the synergistic antimicrobial activity of GSE or GTE with nisin against *L. monocytogenes* in meat model systems (Sivarrooban et al., 2007, 2008; Gadang et al., 2008).

Antimicrobial activity of the plant extracts can be attributed to their action on the bacterial cell membrane (Cowan, 1999). Therefore, using the antimicrobial compounds that have similar action on the bacterial cell wall through multiple hurdle approach could yield synergistic effects. Therefore, the purpose of this study is to investigate the effect of green tea, grape seed extracts, nisin, and EDTA combinations to reduce the chemical antimicrobials such as potassium lactate and sodium diacetate on inhibiting *L.m* in a ready-to-eat hotdog model system.

## MATERIALS AND METHODS

### MATERIALS

Low and high fat chicken and turkey hotdogs were formulated for an *L.m* challenge study. Fresh, boneless, skinless chicken breast meat (2.4 % fat), mechanically separated chicken (24 % fat) (Tyson Foods Inc., Springdale, AR), and mechanically deboned turkey with fat levels of 7 % and 24 % (Cargill Meat Solutions, Springdale, AR) were used in this study. Non-meat ingredients such as salt, sodium tripolyphosphate, dextrose, monosodium glutamate, (Heartland Supply, AR, USA), nitrite (Butcher and Packer Supply, MI, USA), red pepper, black pepper (Eatam Foods Company, NJ, USA), potassium lactate (PURASAL HiPure P, Purac America), and sodium diacetate (Jarchem, NJ) were used in this study. Non-edible casings were used to stuff the emulsified meat (E-Z Peel4 Nojax 30 mm diameter fibrous cellulose casings, Viskase Corp., Willowbrook, IL, USA). Modified oxford selective agar was used to isolate *Listeria monocytogenes* (EMD Chemicals Inc., Gibbstown, NJ, USA). In addition, green tea (Sunphenon<sup>®</sup> 90M-T, Taiyo International, Inc, Minneapolis, MN) and grape seed extract (MegaNatural<sup>®</sup> Gold Grape Seed Extract, Polyphenolics, Madera, CA), and nisin (Profood International, Naperville, IL) were to reduce potassium lactate and sodium diacetate used in hotdog formulations.

### Hotdog preparation

Low fat (5 % fat in RTE product) hotdogs were formulated using ground boneless, skinless chicken breast meat or mechanically deboned turkey meat, whereas the high fat (20 % fat in RTE product) hotdogs were formulated with mechanically separated chicken or turkey meat. Meat block was chopped and ground in a bowl chopper (Type K64V-VA, Seydelman, Germany) and then mixed with non-meat ingredients and varying levels of chemicals (PL-SDA),

plant extracts (GTE-GSE), nisin, and EDTA combinations (Table 4.1), depending on the treatment, to form a homogenous emulsion batter in a bowl chopper. Emulsified meat was placed in a sausage stuffer (Friedrich Dick hand stuffer, 15LTR, Germany) with inedible cellulose casings (30 mm diameter), and slid along the horn of the stuffer. The sausage mixture was stuffed, extruded into 6-inch hotdog links. Hotdogs were placed on cooking sticks in oven (ALKAR-RapidPak, Inc, Model-1000, Wisconsin, USA) at 82.2 °C until the internal temperature reached 73.8 °C to ensure killing of foodborne pathogens in the hotdog. After cooking, hotdogs were cooled by water shower at 25 °C and stored at 4 °C for further studies.

### **Preparation of bacterial suspension**

A loop of frozen (at -70 °C) stock culture (*L.m*) was transferred to brain heart infusion (BHI) broth (10 mL) and incubated (New Brunswick Scientific shaker incubator at 200 rpm; Edison, N.J. USA) at 37 °C for 24 h. After incubating, 10 µL of *L.m* culture was inoculated in 10 mL of fresh BHI, vortexed and incubated at 37 °C for 18 h. The incubated cultures were centrifuged (J2–21 Centrifuge, Beckman, Fullerton, CA, USA) at 25 °C for 10 min to obtain the supernatant and the culture pellets. The pellets were washed twice with phosphate buffer saline (PBS) and resuspended in the volume of PBS that was equal to the original volume of BHI in the culture. Serial dilutions of the bacterial suspension were made to obtain approximately  $10^6$  cfu/mL (colony forming units) for surface inoculation of the hotdog samples.

### **Surface inoculation and plating of the hotdog samples**

Hotdog casings were peeled off in sterile conditions (in a biological safety cabinet) and sliced into samples for surface inoculation as described by Gadang et al. (2008). In this study, we used sliced hotdog samples (1 cm<sup>3</sup>; 1.0 to 1.5 gram) as the surface model system and treated them as an experimental unit (for better control over experimental conditions) for microbial

enumeration. For surface inoculation, hotdog samples were dipped into the *L.m* cultures separately ( $\sim 10^6$  cfu/mL) for 1 min and air dried for 20 min under laminar flowing conditions that enables the bacterial cells to attach to the hotdog samples as described by Over et al. (2010). All hotdog samples (with or without surface inoculation) were transferred to sterile whirlpak bags (7.5 x 12.5cm; 2.25 mil thickness and 276.CC/100 Sq. Inch), vacuum packed (VacMaster-VP 215, Portland, OR, USA), and stored at 4 °C.

### **Microbial evaluation**

Hotdog samples were removed on 0, 4, 8, 12, 16, 21, and 28 days of storage and determined for growth inhibitory activities of treatments. Phosphate buffer saline (PBS at pH 7.0) was added to the bags to make a 10 fold dilution and stomached (Neutec Group Inc.191 masticator; Torrent de l'Estadella, 22 08030 Barcelona, Spain) for 120 seconds to ensure uniform mixing of PBS with inoculated sample and better distribution of the pathogens in the homogenate. Stomached samples were serially diluted with PBS and inoculated (spread plating) on selective agar plates for the colony count enumeration. Inoculated agar plates were incubated at 37 °C for 48 hrs and enumerated to determine the growth inhibition properties of the treatments.

### **Experimental design and statistical analysis**

The experiment design was a split plot where the whole plot was completely randomized with [two meats (chicken and turkey) X two fats (high and low fat) X seven treatment] and the split plot factor was the seven storage times (0, 4, 8, 12, 16, 21, and 28 days) with three replications (three hotdog samples at each sampling time). Analysis of variance was performed using PROC MIXED in SAS<sup>®</sup> version 9.2 (SAS Institute, Cary, NC, USA) and used to determine statistical differences among the main effects and their interactions with a significance

level of  $P < 0.05$ . Significant differences among the least squares growth means ( $P < 0.05$ ) among the treatments were used to identify the effective combination of PL-SD and GTE-GSE. For each storage time (day), growth inhibitions (log cfu/g) were calculated as the difference between mean log count for the control and the mean log count of treatment sample on that particular day.

## RESULTS AND DISCUSSION

Chemical preservatives [Optimal combinations of PL (3.0 %) and SD (0.15 % as determined in Chapter III)] were replaced at varying levels (25 %, 50 %, and 75 %) by natural plant extracts [GTE (0.35 %) and GSE (0.22 %) to determine the effect on growth inhibition of *L.m* on surface inoculated low and high fat chicken and turkey hotdogs. Growth of *L.m* in low and high fat chicken hotdogs formulated with chemical preservatives alone and in varying combinations with plant extracts are presented in Figure 4.1. Low and high fat chicken hotdogs formulated with no antimicrobials (control) supported the growth of *L.m* and spoiled by 28 days (~ 8.5 log cfu/g). Addition of antimicrobials such as chemical preservatives (PL and SD), plant extracts (GTE and GSE), nisin, and EDTA combinations at varying levels achieved growth inhibition of *L.m* (Fig. 4.1). Low and high fat chicken hotdog samples formulated with PL-SD combination (T2: optimal levels - 3.0 % and 0.15 %, respectively, as determined in chapter III) demonstrated the highest growth inhibition (2.45 log cfu/g and 2.62 log cfu/g on 21<sup>st</sup> and 12<sup>th</sup> day of storage respectively) among all the treatments. However, low and high fat hotdogs formulated with PL-SD combination at commercial usage levels (T7: 2.0 % and 0.15 % respectively) had lower growth inhibition of *L.m* (1.65 log cfu/g and 0.80 log cfu/g respectively on 12<sup>th</sup> day of storage). Chicken hotdogs (low and high fat) formulated with plant extracts, nisin, and EDTA alone (T3) exhibited demonstrated growth inhibitions (1.22 log cfu/g) until 8<sup>th</sup> day,

and thereby grew to 8.12 log cfu/g until spoilage (28 days). Chicken hotdogs (low and high fat) formulated with plant extracts replacing PL-SD (increasing order: 25 %, 50 %, 75 %) had no significant difference ( $P > 0.05$ ) in *L.m* growth. However, growth inhibitions of these treatments are similar to the treatments formulated with chemical preservatives alone (T2 or T6) until 8<sup>th</sup> day. Replacement of chemical preservatives by plant extracts at 25 % level (T6: GTE – 0.35 % and GSE- 0.22 %) exhibited desirable sausage characteristics, as the sensory attributes (color, taste, and texture) have minimal effect by the addition of plant extracts. Low and high fat chicken hotdogs of this treatment (T6) demonstrated higher growth inhibition (1.32 log cfu/g and 0.64 log cfu/g respectively) compared to other treatments formulated with both chemical preservatives and plant extracts (T4 and T5: 75 % and 50 % replacement levels). In addition, no significant differences in growth ( $P > 0.05$ ) were found over time (28 days) in hotdogs formulated with commercial usage level of PL-SD combination (T7) and treatments formulated with reduced PL-SD (25 % replacement) and GTE-GSE combination (T6).

Growth of *L.m* in turkey hotdog samples (low and high fat) formulated with no antimicrobials and varying combination levels of chemical preservatives and plant extracts are presented in Figure 4.2. Turkey hotdog samples (low and high fat) formulated with no antimicrobials grew to 8.6 log cfu/g until spoilage (28 days) (Fig 4.2). Maximum growth inhibitions (1.95 log cfu/g) were observed in low and high fat turkey hotdog samples formulated with PL (3.0 %) and SD (0.15 %) (T2). Turkey hotdog samples (low and high fat) formulated with plant extracts, nisin, and EDTA combinations (T3) had demonstrated growth inhibitions until 8<sup>th</sup> day of storage and grew to 8.3 log cfu/g until spoilage. Though not significantly different ( $P > 0.05$ ), treatments formulated with optimal concentration of PL and SD (determined in Chapter III: T2) demonstrated higher growth than commercial usage of PL and SD (T7: 1.40

log cfu/g). Among the treatments formulated with chemical preservatives and plant extracts (T4, T5, T6), partial replacement (25 % level) of chemical preservatives with plant extracts (T6: 1.44 log cfu/g) had demonstrated similar growth inhibitions with treatments formulated with commercial usage levels of PL and SD (T7: 1.45 log cfu/g). In addition, fat level had significant ( $P < 0.05$ ) effect on growth of *L.m* as low fat turkey hotdogs demonstrated higher growth inhibition compared to high fat.

These results demonstrate that partial replacement (25 % level) of chemical preservatives by natural green tea and grape seed extracts along with nisin and EDTA combinations had similar growth inhibition as that of treatments formulated with commercial usage levels of PL (2.0 %) and SD (0.15 %). Though GTE and GSE, along with nisin and EDTA combinations, demonstrated growth inhibitions against *L.m* until the 8<sup>th</sup> day of storage, they may not have been sufficient alone to completely inhibit and extend the shelf life. Therefore, multiple hurdles such as chemical preservatives, synergistic action of GTE and GSE with nisin and EDTA, vacuum packaging, and storage temperatures contribute to decontaminate and extend the shelf life. Theivendran et al. (2006) have shown synergistic inhibitory effects of GSE (1%) or GTE (1%) in combination with nisin (10,000 IU/mL) than plant extracts alone against starving *L. m* in PBS medium incubated at 37 °C. These synergistic activities may be due to facilitating the diffusion of major phenolic compounds in GTE (epicatechin, caffeic, benzoic, and syringic acid) or GSE (epicatechin, catechin, genistic, and syringic acid) through the pores formed in the pathogenic cell membrane caused by the activity of nisin (Theivendran et al., 2006). Furthermore, application of GSE (1%) and nisin (6400 IU/mL) combinations, through soy protein film coating, inhibited *L.m* populations to undetectable levels (minimum detection limit was 100 cfu/g) for complete fat turkey frankfurter formulations (21% fat) when stored at both 4 °C and 10

°C (Thievendran et al., 2006). Antimicrobial effects of GTE and GSE combinations in full fat frankfurter are due to the presence of carrier system i.e. soy protein isolate film that helps in interacting with the pathogen on the surface of the hotdog until it inhibits to undetectable levels. Addition of grape seed and green tea extracts increased the shelf life of raw patties and other meat products stored under retail display conditions (Banon et al., 2007). Both the extracts (GTE and GSE) have an antibacterial effect in in vitro conditions (Ahn et al., 2004). Incorporating major phenolic constituents in GTE or GSE along with nisin can demonstrate changes to the morphology and internal structures of *L.m* cells using transmission electron microscopic studies (Sivaroooban et al., 2008).

Differences in the results in the model system can be explained by the interactions and complexity of the system. Natural plant extracts that have potential antimicrobial components may be less effective in food systems due to their interactions with lipids and proteins present in the food, which may ultimately reduce the antimicrobial activity of these compounds. Though combination of GTE and GSE along with nisin showed significant growth inhibitions of *L.m*, efficiency of this combination may be further enhanced when hotdogs are challenged with lower inoculation levels.

## **CONCLUSIONS**

Green tea and grape seed extracts are natural alternatives that demonstrated growth inhibition of *L.m* in hotdog (chicken and turkey hotdogs). Fat level has significant effect on growth as indicated by higher inhibitions in low fat hotdog (chicken and turkey; 1.45 log cfu/g) samples than high fat samples (0.80 log cfu/g). Conventional chemical preservatives can be partially replaced (25 % level) by natural plant extracts such as GTE and GSE to reduce the

chemical preservatives, and therefore meet consumer demand for meat products with minimal synthetic food additives.

## REFERENCES

- Ahn, J., Grun, I.U. and Fernando, L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J Food Sci*, 67, 1364–1369.
- Ahn, J., Grun, I.U. and Mustapha, A. 2004. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J Food Prot*, 67, 48–55.
- Banon, S., Diaz, P., Rodriguez, M., Garrido, M. D. and Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Sci*, 77, 626–633.
- Cannarsi, M., Baiano, A., Sinigaglia, M., Ferrara, L., Baculo, R. and Del Nobile, M.A. 2008. Use of nisin, lysozyme and EDTA for inhibiting microbial growth in chilled buffalo meat. *Int J Food Sci Technol*, 43, 573–578.
- CDC. 2010. Preliminary FoodNet Data on the incidence of infection with pathogens transmitted commonly through food—10 States, 2009. *Morb Mortal Wkly Rep*, 59, 418-422.
- Cowan, M. M. 1999. Plant products as antimicrobial agents clinical microbiology reviews, *Am Soc Microbiol*, 12, 564–582.
- Cox, S.D., Mann, C.M. and Markham, J.L. 2001. Interactions between components of the essential oil of *Melaleuca alternifolia*. *J Appl Microbiol*, 91, 492–4977.
- Beuchat, L.R., Brackette, R.E. and Doyle, M.P. 1994. Lethality of carrot juice to *Listeria monocytogenes* as affected by pH, sodium chloride, and temperature. *J Food Prot*, 57, 470–474.
- Bozariis, I.S., Adams, M.R., 1999. Effect of chelators and nisin produced in situ on inhibition and inactivation of gram negatives. *Int J Food Microbiol*, 53, 105–113.
- Davidson, P.M. and Naidu, A.S. 2000. Phyto-phenols. In A. S. Naidu (Ed.), Natural food antimicrobial systems (pp. 265–294). Boca Raton, FL, USA: CRC Press LLC.
- Davidson, P.M. and Harrison, M.A., 2002. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. *Food Technol*, 56, 69–78.
- Elgayyar, M., Draughon, F. A., Golden, D. A. and Mount, J.R. 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J Food Prot*, 64, 1019–1024.
- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C.M. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *J Food Sci*, 73, 389–394.

- Ganesh, V., Hettiarachchy, N.S. and Ravichandran, M. 2010. Electrostatic sprays of food-grade acids and plant extracts are more effective than conventional sprays in decontaminating *Salmonella* Typhimurium on spinach. *J Food Sci*, 75, 74–79.
- Geissman, T.A. 1963. Flavonoid compounds, tannins, lignins and related compounds, p. 265. In M. Florkin and E. H. Stotz (ed.), *Pyrrrole pigments, isoprenoid compounds and phenolic plant constituents*, vol. 9. Elsevier, New York, N.Y.
- Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T. 1993. Bactericidal catechins damage the lipid bilayer. *Biochemica et Biophysica Acta*, 1147, 132–136.
- Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. 2003. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res Int*, 36, 117–122.
- Juneja, V., Hwang, C.A. and Freidman, M. 2010. Thermal inactivation and post treatment growth during storage of multiple *Salmonella* serotypes in ground beef as affected by sodium lactate and oregano oil. *J Food Sci*, 75, 1–6.
- Over, K.F., Hettiarachchy, N.S., Johnson, M.G. and Davis, B. 2009. Effect of organic acids and plant extracts on *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium in broth culture model and chicken meat systems. *J Food Sci*, 74, 515–521.
- Over, K.F., Hettiarachchy, N.S., Perumalla, A.V.S., Johnson, M.G., Meullenet, J.F., Dickson, J.S., Holtzbauer, M.J., Niebuhr, S.E. and Davis, B. 2010. Antilisterial activity and consumer acceptance of irradiated chicken breast meat vacuum-infused with grape seed and green tea extracts and tartaric acid. *J Food Sci*, 75, 455-461.
- Perumalla, A.V.S., Hettiarachchy, N.S., Ravichandran, M., Slavik, M. and Kumar, G.S. 2010. Grape seed extract and malic acid effectively inhibit the growth of *Campylobacter jejuni* in broth culture. *J Food Prot*, Suppl. 64(P1-28).
- Shoko, T., Soichi, T., Megumi, M. M., Eri, F., Jun, K. and Michiko, W. 1999. Isolation and identification of an antibacterial compound from grape and its application to foods. *Nippon Nogeikagaku Kaishi*, 73, 125–128.
- Sivaroban, T., Hettiarachchy, N.S. and Johnson, M.G. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10 °C. *J Food Prot*, 70, 1017–1020.
- Sivaroban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. *Food Res Int*, 41, 781–785.
- Theivendran, S., Hettiarachchy, N.S. and Johnson, M.G. 2006. Inhibition of *Listeria monocytogenes* by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *J Food Sci*, 71, 39–44.

USDA, Economic Research Service, 2010. Foodborne illness cost calculator. Accessed at <http://www.ers.usda.gov/Data/FoodborneIllness/>, March 2011.

Xia, E.Q., Deng, G.F., Guo, Y.J. and Li, H.B. 2010. Biological activities of polyphenols from grapes - Review. *Int J Mol Sci*, 11, 622–646.

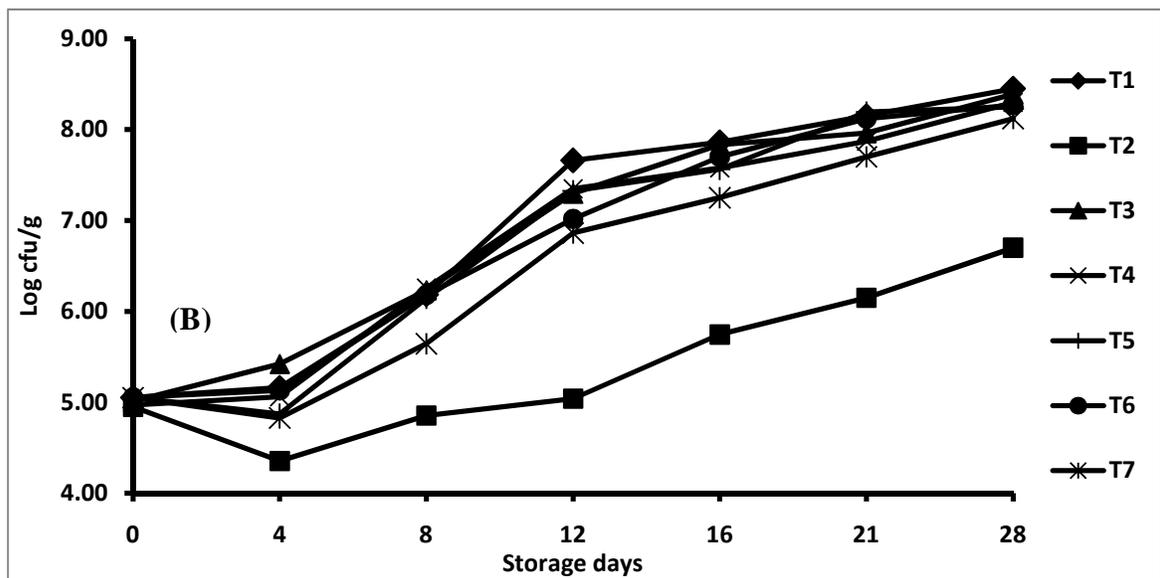
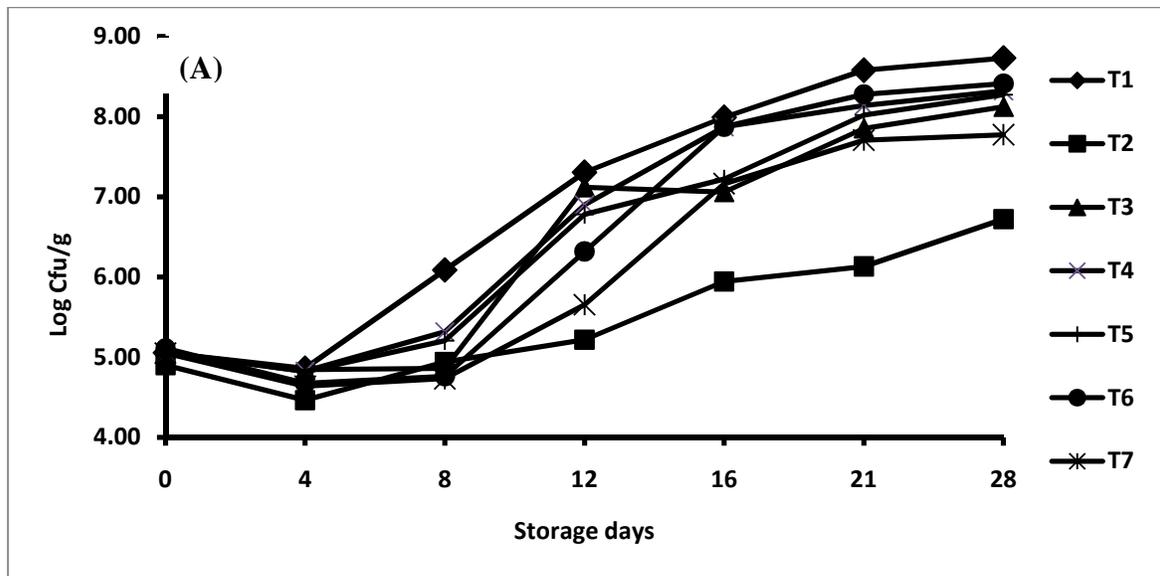
Yilmaz, Y. 2006. Novel uses of catechins in foods. *Trends in Food Sci Technol*, 17, 64–71

**Table 4.1** Treatments showing various combination levels of potassium lactate (PL) and sodium diacetate (SD) alone and in combination with green tea extract (GTE), grape seed extract (GSE), nisin, and EDTA used in this study.

Treatment	Chemical/plant extracts	Concentration (%) <sup>1</sup>					
		PL	SDA	GTE	GSE	Nisin	EDTA
T1	Control (No antimicrobial)	X	X	X	X	X	X
T2	PL + SD	3.0	0.10	X	X	X	X
T3	GTE + GSE	X	X	1.4	0.9	X	X
T4	25 % (PL+SD): 75 % (GSE+GTE) + Nisin + EDTA	0.75	0.025	1.05	0.675	0.03	0.3
T5	50 % (PL+SD) + 50 % (GSE+GTE) + Nisin + EDTA	1.5	0.05	0.7	0.45	0.03	0.3
T6	75 % (PL+SD) + 25 % (GSE+GTE) + Nisin + EDTA	2.25	0.075	0.35	0.225	0.03	0.3
T7	PL + SD	2.0	0.15	X	X	0.03	0.3

<sup>1</sup>Based on the percent weight of the formulation

X = Not incorporated in the formulation.



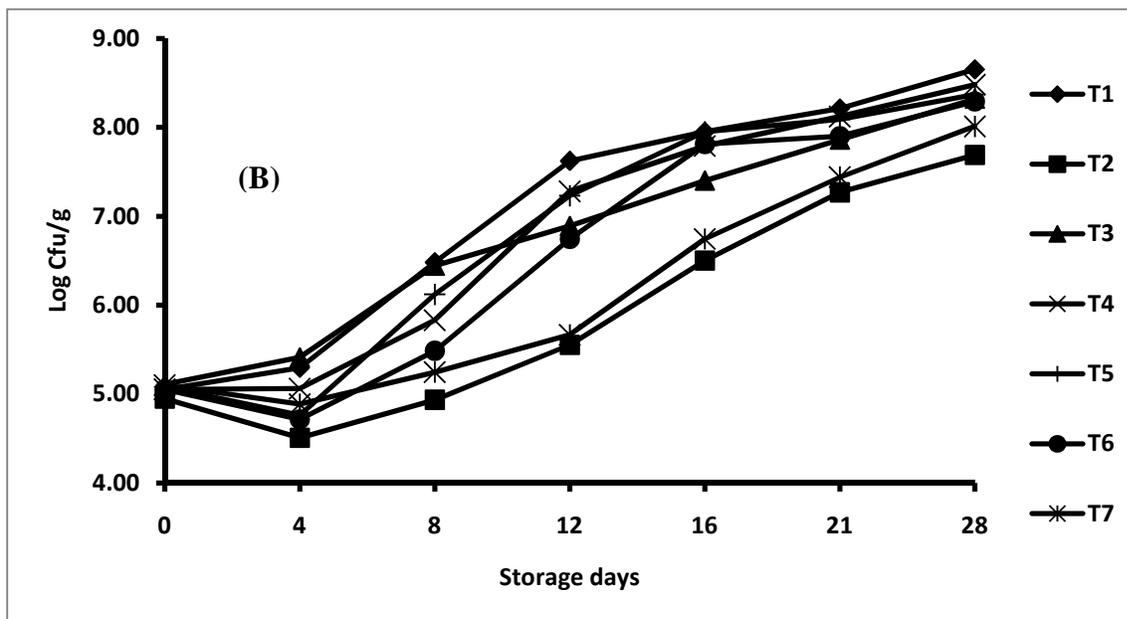
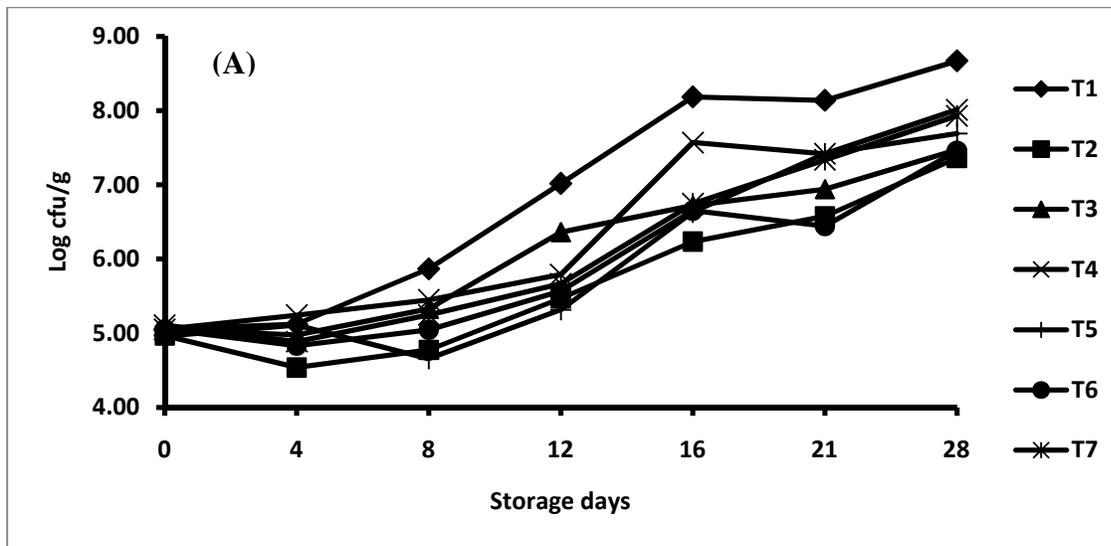
**Figure 4.1** Effect of reducing potassium lactate (PL) and sodium diacetate (SDA) by plant extracts, nisin, and EDTA against *Listeria monocytogenes* in (A) low and (B) high fat chicken hotdogs.

Data are mean log numbers on the 0, 4, 8, 12, 16, 21, and 28 days and error bars indicate the standard error of means from three replications.

(T; PL, SDA): T1: Control (no antimicrobials); T2: PL-SD; T3: GTE-GSE; T4: 25 % (PL-SD): 75 % (GSE+GTE)-Nisin-EDTA; T5: 50 % (PL+SD)-50 % (GSE+GTE)-Nisin-EDTA; T6: 75 % (PL-SD)-25 % (GSE-GTE)-Nisin-EDTA; T7: 2.0 % PL + 0.15 %SD).

LSD to compare means to determine significant differences ( $P < 0.05$ ) at same meat or fat or PL-SD- GTE-GSE combinations = 0.21

LSD to compare means to determine significant differences ( $P < 0.05$ ) at different meat or fat or PL- SD- GTE-GSE combinations = 0.19



**Figure 4.2** Effect of reducing potassium lactate (PL) and sodium diacetate (SDA) by plant extracts, nisin, and EDTA against *Listeria monocytogenes* in (A) low and (B) high fat turkey hotdogs.

Data are mean log numbers on the 0, 4, 8, 12, 16, 21, and 28 days and error bars indicate the standard error of means from three replications.

(T; PL, SDA): T1: Control (no antimicrobials); T2: PL-SD; T3: GTE-GSE; T4: 25 % (PL-SD): 75 % (GSE+GTE)-Nisin-EDTA; T5: 50 % (PL+SD)-50 % (GSE+GTE)-Nisin-EDTA; T6: 75 % (PL-SD)-25 % (GSE-GTE)-Nisin-EDTA; T7: 2.0 % PL + 0.15 %SD).

LSD to compare means to determine significant differences ( $P < 0.05$ ) at same meat or fat or PL-SD- GTE-GSE combinations = 0.21

LSD to compare means to determine significant differences ( $P < 0.05$ ) at different meat or fat or PL-SD- GTE-GSE combinations = 0.19

## CHAPTER V

### **EFFECT OF PARTIAL REPLACEMENT OF POTASSIUM LACTATE AND SODIUM DIACETATE BY NATURAL GREEN TEA AND GRAPE SEED EXTRACTS AND POST-PACKAGING THERMAL TREATMENT ON THE GROWTH OF *LISTERIA MONOCYTOGENES* IN HOTDOG MODEL SYSTEM**

#### **ABSTRACT**

Low (5 %) and high fat (20 %) chicken and turkey hotdogs were formulated in three groups: no antimicrobials (control), chemical preservatives alone (potassium lactate and sodium diacetate), and partial replacement of chemical preservatives by green tea and grape seed extracts, surface inoculated ( $\sim 10^3$  log cfu/g) with *Listeria monocytogenes*, subjected to with or without heat treatment (65 °C for 104 s), and stored at 4 °C to determine the growth of *L. monocytogenes* until spoilage (28 days). Results have shown that chemical preservatives alone are not sufficient to inhibit the growth of *L. monocytogenes* as it increased by at least 4.0 log cfu/g by 28 days until spoilage. Maximum growth inhibitions ( $\sim 2.0$  log cfu/g) were observed in the treatments having chemical preservatives and plant extracts regardless of the meat and fat type. Furthermore, plant extracts along with chemical preservatives demonstrated additional inhibitory effects on the growth of *L. monocytogenes* survivors followed by post-packaging heat treatment in chicken hotdog samples. Fat level had a significant effect ( $P < 0.05$ ) in the chicken hotdog samples subjected to heat treatment as high fat samples are more resistant to heat treatment compared to low fat samples. Results demonstrated that natural green tea and grape seed extracts can partially replace the chemical preservatives, and, in addition, enhance the antilisterial activities in this model system when combined with heat treatment.

## INTRODUCTION

*Listeria monocytogenes* is a formidable post-processing pathogen contaminant on ready-to-eat (RTE) meat products, including hotdogs. Contamination of hotdogs with *L. monocytogenes* represents a major public health risk, and therefore demands effective elimination or reduction at the pre- or post-packaging level.

Incorporating chemical preservatives such as lactates and diacetates in combination can control the growth of *L. monocytogenes* (Mbandi & Shelef, 2002; Seman et al., 2002; Sommers et al., 2003; Knight et al., 2007). Maximum permissible levels of lactates and diacetates used in meat formulations are 3.0 % and 0.25 %, respectively (FSIS, 2000). However, these levels alone are not sufficient to inhibit the growth of *L. monocytogenes* to minimal levels through the shelf life of the product (Wederquist et al., 1994; Bedie et al., 2001). Therefore, additional hurdles such as natural plant extracts (Sivaroooban et al., 2007, 2008; Bisha et al., 2010) and pre- or post-packaging thermal treatments (steam, hot water, irradiation) (Roering et al., 1998; Murphy et al., 2005) are expected to enhance the total antilisterial effects in the products. Better control of pathogens can be achieved when such hurdles are applied in combination (Murphy et al., 2006; Juneja et al., 2010).

There is an increasing trend in the consumer preference for fewer synthetic preservatives by replacing them with natural alternatives that would improve safety, add nutraceutical value, and provide health benefits (Vigil et al., 2005). In recent years, phenolic-rich extracts such as green tea (GTE) and grape seed extracts (GSE) have gained popularity as they are generally recognized as safe (GRAS) food additives with antimicrobial and anti-oxidant properties (Perumalla and Hettiarachchy, 2011). Green tea extract is a dried leaf extract obtained from cultivated tea plant (*Camellia sinensis*), rich in catechins and flavonoids (Kajiya et al., 2004).

Grape seed extract, a value-added by-product of the grape and wine juice industry is rich in flavonoids and phenolics (Lau and King, 2003). Both GTE and GSE have demonstrated antimicrobial properties against foodborne pathogens including *L. monocytogenes* in broth as well as meat model systems (Ahn et al., 2004; Sivarooban et al., 2008; Gadang et al., 2008). Furthermore, GTE and GSE have been used in various antimicrobial strategies including multiple hurdle components to provide enhanced protection (Perumalla and Hettiarachchy, 2011). However, the majority of the previous studies applied these plant extracts either by dipping or spraying on food surfaces rather than adding directly to the formulation (Sivarooban et al., 2008; Bisha et al., 2010).

One of the most common and efficient methods in processing conditions to control the contamination levels of *L. monocytogenes* is heat application (Porto et al., 2004). Application of post-packaging thermal treatments demonstrated successful elimination of *L. monocytogenes* on the surface of RTE meat and poultry products (McCormick et al., 2005; Sommers et al., 2008). Inclusion of multiple hurdles (storage temperature, vacuum packaging, chemical preservatives, plant extracts, and heat treatment) in the food system that have different mode of action(s) are more effective in pathogen inhibition (Ricke et al., 2005). Therefore, in pursuit of consumer trends, and to control the contamination risks, a novel strategically combinations consisting of GSE, GTE, potassium lactate (PL), sodium diacetate (SD), and post-packaging heat treatment may provide promising results.

Antimicrobial effects of additives against the growth of *L. monocytogenes* depend on processing (pH,  $a_w$ , moisture, fat, nitrite, and salt content) and storage (temperature and package atmosphere) conditions (Glass and Doyle, 1989; Grau and Vanderlinde, 1992; Nebrink et al., 1999; Juncher et al., 2000). In addition, thermal resistance of the pathogen is also affected by

meat species, experimental conditions, and inoculum treatment before heating and fat content (Mackey et al., 1990; O'Bryan et al., 2006). Among these factors, fat content plays an important role in offering thermal protection, and in turn gives more resistance to the product, ultimately affecting thermal lethality of the pathogen (Murphy et al., 2004). Furthermore, in biphasic foods such as hotdogs (oil-in-water emulsion), fat content may influence pathogen growth by its tendency to redistribute the chemical components (antimicrobials in particular) between food phases. As the undissociated form of organic acid is lipophilic, less of the undissociated acid or antimicrobial may localize in the aqueous phase and hence can affect their efficacies (Leo et al., 1971).

This first of a kind study involves novel strategically application of GSE and GTE (within the organoleptic acceptable levels) in poultry meat formulations along with potassium lactate and sodium diacetate that satisfies increased consumer demand for natural products. Our main objective of this study was to investigate the effect of partial replacement of PL and SD by natural GTE and GSE against the growth of *L. monocytogenes* in a meat model system until spoilage. Furthermore, combinations of these antimicrobial agents in conjunction with heat treatment were also investigated in a laboratory setting to determine the total antilisterial effects.

## **MATERIALS AND METHODS**

### **Materials**

Low (5 %) and high (20 %) fat chicken hotdogs were formulated using boneless, skinless, chicken breast and mechanically separated chicken meat (Tyson Foods Inc., Springdale, AR, USA) respectively, while turkey hotdogs (low and high fat) were formulated using mechanically separated meat (Cargill Meat Solutions, Springdale, AR, USA). Non-meat ingredients included salt, nitrite, sodium tripolyphosphate, dextrose, monosodium glutamate, (Heartland Supp. Co,

AR, USA), red pepper, black pepper (Eatem Foods Company, NJ, USA), potassium lactate (PL), (PURASAL HiPure P, Purac America), and sodium diacetate (SD) (Jarchem, NJ, USA). Non-edible casings were used to stuff the emulsified meat (Casings: 30 mm diameter fibrous cellulose casings; E-Z Peel4 Nojax, 30-84 4STR clear, Viskase Corp., Willowbrook, IL, USA). Natural plant extracts including green tea extract (Sunphenon® 90M-T, Taiyo International, Inc, Minneapolis, MN) and grape seed extract (MegaNatural® Gold Grape Seed Extract, Polyphenolics, Madera, CA) were used. Modified Oxford *Listeria* selective agar (EMD Chemicals Inc., Gibbstown, NJ, USA) was used to isolate *Listeria monocytogenes*.

## **Methods**

### **Level of antimicrobials and heat treatment intervention parameters**

Concentration levels of antimicrobial agents (PL, SD, GSE and GTE) and heat treatment intervention (time and temperature combination) were selected (preliminary study in our laboratory) while considering their practical application in meat products such as hotdogs without affecting the organoleptic attributes of the product. An initial study was conducted to investigate the level of replacement of chemical preservatives by plant extracts without compromising the product organoleptic attributes and found to be at 25 % (i.e. chemical preservatives: plant extracts - 75:25). Presently, PL ( $\leq 2$  %) and SD (0.15 %) are the chemical preservatives that are extensively used in commercial hotdog formulations, and therefore are considered as a reference treatment to compare the effect of partial replacement of PL and SD by GTE and GSE on the growth of *L. monocytogenes*. Optimum combination levels of GTE and GSE to inhibit the growth of *L. monocytogenes* in a surface inoculated hotdog study (vacuum packed, stored at 4 °C) were determined to be 1.4 % and 0.9 % (data not shown). In preliminary heating trials (broth culture), the temperature (°C) and time (sec) combinations included were

62.5 (145), 65 (104), 67.5 (24), 70 (12), and 80 (6) in broth culture studies. Based on the heat inactivation, surface texture, and purge losses, a combination of 65 °C for 104 s was chosen.

### **Preparation of chicken and turkey hotdogs**

Non-meat ingredients in the basic (no antimicrobials) hotdog formulation (% of total weight in the formulation) included ice (10), salt (1.25), sodium tripolyphosphate (0.4), dextrose (0.2), sodium nitrite (0.0156), red pepper (0.12), black pepper (0.15), and monosodium glutamate (0.02). Treatments with chemical preservatives alone consisted of PL (2 %) and SD (0.15 %) while the treatments with partial replacement (at 25 % level) of chemical preservatives with plant extracts consisted of PL (1.5 %), SD (0.11 %), GTE (0.35 %), and GSE (0.22 %).

Meat block, non-meat ingredients, and antimicrobials (as per the treatment) were mixed and emulsified in a bowl chopper (Type K64V-VA, Seydelman, Germany) to form a homogenous meat batter. Meat batter was extruded through a stuffer (Friedrich Dick hand stuffer, 15 LTR, Germany) into inedible cellulose casings, manually pinched, and twisted into 6-inch hotdog links. Raw hotdog links were cooked in an oven (Alkar-RapidPak, Inc., Model 1000, Lodi, WI) at 82.2 °C until the internal temperature of the hotdogs reached 73.8 °C. After cooking, the hotdogs were showered with water at 25.5 °C and stored at 4 °C for further studies.

### **Preparation of bacterial suspension**

Frozen stock (at -70 °C) of *Listeria monocytogenes* (strain V7, serotype ½ a; FDA isolate) provided by Centre for Food Safety Laboratory (Fayetteville, AR) was transferred to BHI broth (10 mL) and incubated (New Brunswick Scientific agitating incubator at 200 rpm; Edison, N.J. USA) at 37 °C for 24 h. About 10 µL of *L. monocytogenes* culture was inoculated into 10 mL of fresh BHI, vortexed and incubated (200 rpm agitation) at 37 °C for 18 h. Following incubation, the cultures were centrifuged (J2–21 Centrifuge, Beckman, Fullerton,

Calif., U.S.A.) at 25 °C for 10 min to obtain the supernatant and the culture pellets. The pellets were washed twice with phosphate buffer saline (PBS) and resuspended in the volume of PBS that was equal to the original volume of BHI in the culture. Bacterial suspension was serially diluted to obtain about  $10^4$  cfu/ml for surface inoculation study.

### **Surface inoculation of hotdogs**

Hotdogs were sliced through the thickness (2.8 cm) to make samples (medallion shaped, approximately 10 g) and used for surface inoculation. A total of 504 hotdog samples [two meat types (chicken and turkey) X 2 fat levels (low and high) X 6 treatments including control X 7 sampling days X 3 replications] were surface inoculated. We used hotdog samples rather than full hotdog for better control on the experimental conditions (surface inoculation, vacuum packaging, and heat treatment at the start of the experiment), based on the number of samples and treatments as demonstrated by previous studies (Lungu et al., 2005 a, b; Theivendran et al., 2006; Sivarooban et al., 2007). Hotdog samples were dipped into the *L. monocytogenes* culture ( $\sim 10^4$  cfu/ml) for 1 min followed by air-drying for 20 min under the laminar hood for bacterial cells attachment to the hotdog surface to achieve a final load of approximately 3-log cfu/g. After drying, each inoculated hotdog sample was transferred to a sterile whirlpak bag (7.5 x 12.5cm; 2.25 mil thickness and 276.CC/100 sq. inch), vacuum packed (VacMaster-VP 215, Portland, OR, USA), and either stored at 4 °C or heat-treated, followed by cooling and storage at 4 °C.

### **Post-packaging thermal treatment**

Equipment used for post-packaging thermal treatment included a water bath (37.5 x 20 x 9.5 inches) (GCA Precision Scientific, Model- Thelco 186, Chicago, IL), heating units (2) with water re-circulator ability (MGW Lauda MS), and a fabricated wire rack (to support and keep the vacuum packed hotdog samples immersed in the water bath by securing the two ends with a

binder clip) (Fig. 1). Two water-heating units with circulating systems were placed at each side of the water bath to minimize the cold spots during heat treatment. A digital temperature probe (thermocouple) was used to monitor the water temperature. Three heat treatment trials for three different treatments including replicates for each meat (chicken and turkey) and fat type (low and high) (12 trials) were conducted in the hot water bath at 65 °C for 104 s. After heating, the hotdog samples were removed from the wire-rack and placed in the ice bath for 5 min to minimize further thermal effects, and stored at 4 °C for further studies. Non-inoculated vacuum-packed hotdog samples were used (kept at random places on the wire rack) to monitor the come-up and come-down times.

### **Microbiological analyses**

Experiment was conducted until the samples were spoiled (based on visual appearance, softening and odor) for 28 days. Hotdog samples were observed on 0, 4, 8, 12, 16, 21, and 28 days from surface inoculation to determine the growth of *L. monocytogenes*. For bacterial enumeration, we used a 10-fold dilution of hotdog sample with PBS solution made in stomacher bags to form a homogenate. The homogenate was serially diluted with PBS and spread on modified oxford agar plates with selective supplement, and incubated at 37 °C for 48 h to enumerate colony counts of *L. monocytogenes*.

### **Experimental design and statistical analyses**

The experiment design was a split plot where the whole plot was completely randomized with [two meats (chicken and turkey) X two fats (high and low fat) X six treatments] and seven storage times (0, 4, 8, 12, 16, 21, and 28 days) with three replications (three hot dog samples at each sampling time). Samples that observed no growth following heat treatment and over storage time (e.g. low and high fat turkey) were not included in the analyses. Analysis of variance was

performed using PROC MIXED in SAS® version 9.2 (SAS Institute, Cary, NC, USA) and used to determine statistical differences among the main effects and their interactions at a significance level of  $P < 0.05$ . For each storage time (day), growth inhibitions/reductions (log cfu/g) were determined as the difference between mean log cfu/g count of the control and the mean log cfu/g count of treatment sample (PL+SD or PL+SD+GTE+GSE combination) on that particular day.

## **RESULTS AND DISCUSSION**

### **Effect of antimicrobials in the hotdog formulation on the growth of *L. monocytogenes***

Hotdogs formulated with lactates and diacetates alone, or in combination with plant extracts, had no significant ( $P > 0.05$ ) effect on the pH and water activity regardless of meat and fat (Table 5.1). Low and high fat chicken and turkey control samples (no antimicrobial and no heat treatment) supported the growth of *L. monocytogenes* ( $> 7.0$  log cfu/g) until spoilage (28 days of storage at 4 °C). Low and high fat chicken hotdogs formulated with chemical preservatives at commercial usage levels (2.0 % PL and 0.15% SD combination) also permitted the growth of *L. monocytogenes* from day of surface inoculation until spoilage by 28 days (8.3 log cfu/g and 9.05 log cfu/g respectively). Similar growth trends were observed in low and high fat turkey hotdogs until spoilage by 28 days (9.11 log cfu/g and 6.91 log cfu/g respectively). These growth trends demonstrate that, alone, the levels of PL and SD combination used in this study are not sufficient to inhibit the growth of *L. monocytogenes* and thereby reduce shelf life.

### **Effect of chemical preservatives and plant extracts combination on the growth of *L. monocytogenes***

One of our primary goals was to achieve enhanced growth inhibition of *L. monocytogenes* by partially replacing the commercial levels of PL and SD with GSE and GTE. Studies conducted in our laboratory (not presented here) demonstrated that hotdog formulations with plant extracts alone are not sufficient to inhibit the growth of *L. monocytogenes*. Therefore,

hotdogs were formulated with partial replacement of chemical preservatives (1.5 % PL and 0.15 % SD) by plant extracts (0.35 % GTE and 0.22 % GSE). In chicken and turkey hotdog samples, treatments formulated with the partial replacement of chemicals with plant extracts have demonstrated significant ( $P < 0.05$ ) lower growth when compared to the treatments formulated with chemical preservatives alone (Fig 5.2 A&B, 5.2 C&D).

Using green tea and grape seed extracts in food applications to improve safety and shelf life has gained popularity in recent years (Perumalla and Hettiarachchy et al., 2011). Previous studies have reported the antimicrobial activities of either GTE or GSE alone or in combination with other antimicrobial agents in various model systems (frankfurters, patties, spinach, and tomato) by dipping and spraying (Gadang et al., 2008; Bisha et al., 2010; Ganesh et al., 2010). Thievendran et al., (2006) reported that application of GSE (1 %) or GTE (1 %) via soy protein film coating on full fat turkey franks reduced *L. monocytogenes* population (2.0 log cfu/g reductions). Similar results were also observed when GSE (0.5 %) was applied on whey protein isolate-coated turkey franks (Gadang et al., 2008). These findings reported log reductions as they were applied through an edible film coating (soy protein or whey) as a carrier agent for antimicrobials to provide enhanced interactions with the surface inoculated pathogens. Differences in the results observed in this study can be attributed to the mode of application of these plant extracts.

Catechins and phenolic acids present in both extracts (GTE and GSE) have antimicrobial activities that can cause changes in morphology and internal structure of the *L. monocytogenes* cell membrane, and adhesion capability to proteinaceous component of the cell membrane to inhibit the growth of *L. monocytogenes* (Sivarooban et al., 2008). Mode of action of lactates and diacetates can be attributed to their ability in intracellular acidification by protonation (Miller

and Acuff, 1994; Hunter and Segal, 1973). It was proposed that phenolic metabolites and lactate radicals behave as proline analogs that can inhibit proline oxidation by proline dehydrogenase at plasma membrane level in prokaryotic bacterium cells such as *L. monocytogenes* (Lin et al., 2005; Apostolidis et al., 2008). Therefore, synergistic inhibition of *L. monocytogenes* by the combination of phenolic-rich phytochemicals and organic acid salts can be expected by proline metabolism regulation. Another possible explanation of enhanced inhibition in treatments with chemical preservatives and plant extracts was due to the multiple hurdles that cause *L. monocytogenes* to spend more energy, thus extending its lag phase.

### **Effect of heat treatment on the growth of *L. monocytogenes***

Heat treatment of vacuum-packed hotdogs in the hot water bath (65 °C for 104 sec) inhibited or killed the initial inoculum levels (~ 3.0 log cfu/g) in all the samples regardless of meat, fat, and antimicrobials in the formulation. However, in low and high fat chicken hotdogs formulated with no antimicrobials, and PL and SD combination alone, the injured cells recovered on day 16 and 12, and grew to 6.75 and 5.5 log cfu/g by 28 days (Fig. 5.2 A&B). These results demonstrate that post-packaging thermal treatments alone could not completely inhibit the growth of *L. monocytogenes* in chicken samples, but may have interacted with chemical preservatives and (or) plant extracts to extend the recovery time and retard the growth. In turkey samples (low and high fat), heat treatment had completely inhibited the growth of *L. monocytogenes* in all the samples suggesting that the type of meat may also play an important role in contributing the pathogen inactivation (Doyle et al., 2001). In heat-treated samples (chicken and turkey) containing PL and SD alone and in combination with plant extracts, the average populations of *L. monocytogenes* on day 28 of storage was 5.5 log cfu/g and no

detectable levels compared to 8.35 and 7.4 log cfu/g in non-heated samples (Fig 5.2 A&B, 5.2 C&D).

These results indicated that the post package thermal treatments generally cause restrictive heat injury to *L. monocytogenes* based on the formulation differences (meat and fat), though to an extent that survivors could not recover and grow on the selective media. However, the positive contribution of the post-packaging thermal treatments exhibited the lowest growth as well as initial pathogen reductions during contamination. In addition, hotdogs formulated with chemical preservatives and plant extracts have demonstrated more antilisterial effects than chemicals preservatives alone.

Several studies have been conducted on post-packaging thermal treatments on RTE meat and poultry products, such as hotdogs (Murphy et al., 2005), deli meats (Gande and Muriana, 2003; Muriana et al., 2004), and further processed products (Murphy et al., 2003), both alone and in combination with various antimicrobial agents that had successfully demonstrated the inhibition of post-processing contamination of *L. monocytogenes*. Thermo tolerance of *L. monocytogenes* depends on various factors including sensitivity to stress factors such as heat, acid, oxygen conditions of that particular pathogen strain, pre-inoculation growth conditions, and composition of the heating menstrum (Doyle et al., 2001). Heat resistance is also affected by the interactions between salt, pH, nitrite, and phosphate levels (Juneja and Eblen, 1999). Among these, salts play an important role in increasing heat resistance due to their ability to decrease the water activity ( $a_w$ ) and increase solute concentration. Schultze et al. (2006) also reported that incorporating sodium lactate and sodium diacetate in the frankfurter slurries had a significant ( $P < 0.05$ ) effect on increasing the heat tolerance. In this study, partial replacement of lactate and diacetate salts (reduced salt concentration) with plant extracts could have reduced the heat

resistance of *L. monocytogenes* and further improved the inhibitory effect as indicated by more growth inhibitions in the treatments with reduced chemical preservative salts i.e. replaced by plant extracts than treatments having chemical preservatives alone in all meat and fat type hotdogs. Results observed in this study are consistent with the findings reported by Samelis et al. (2002) that post-process thermal interventions (75 °C or 80 °C for 30 to 90 s) in frankfurters did not inhibit *L. monocytogenes* for more than 10 to 20 days. Contrasting results in comparison to previous studies can be attributed to the differences in the strain used, dose of inocula and its physiological state, type of inoculation (surface, distributed in the emulsion), difference in the proximate composition, and come-up times required to meet target temperatures (Hansen and Knochel, 2001; Juneja et al. 2002; Poysky et al., 1997).

#### **Effect of fat content of the formulation on *L. monocytogenes* growth followed by post-packaging heat treatment**

Level of fat in the hotdog also had variable effects depend upon heat treatment, meat type and on growth inhibition of *L. monocytogenes*. High fat chicken hotdogs had significantly ( $P < 0.05$ ) higher growth than low fat hotdogs both in heat and non-heat treated treatment (Fig 5.2 A&B, 5.2 C&D). In non-heat treated turkey hotdogs, low fat hotdogs had significantly ( $P < 0.05$ ) higher growth than high fat samples regardless of the type of antimicrobial in the formulation. This may have been due to variation in meat species and presence of high concentration of lipid oxides and peroxides that may provide toxic activities to *L. monocytogenes* (Doyle et al., 2001). No survivors were observed in the turkey samples (low and high) subjected to heat treatment. Previous studies have demonstrated that the heat resistance of bacteria increases with increasing fat content (Juneja et al., 2001; Smith et al., 2001). Amount of fat in the meat mixture before cooking also influences the texture of the sliced hotdog surface, as fat helps in forming good emulsion and binding properties with meat protein.

Results of this study demonstrated that combinations of chemical preservatives (PL and SD) and plant extracts (GTE and GSE) provided better inhibition against *L. monocytogenes* than lactates and diacetate combination alone. Furthermore, application of heat treatment in conjunction with the antimicrobials in the formulation has provided enhanced antilisterial effects in the system. These effects are consistent with the concept that multiple hurdles applied for *L. monocytogenes* inhibition were more effective than single form by causing simultaneous and variable damage to bacterial cells (Leistner, 2000). Differences in results observed between experiments done in water baths and actual processing conditions can be expected. Increasing the time and temperature of the heating conditions may offer more log reductions; however, we should always consider the product quality such as yield, texture, and juiciness as high temperature could compromise such attributes. In addition to the factors that affect the product safety and quality discussed above, processors should also consider other thermal inactivation guidelines such as the type of product and the pathogen strain. Further studies including a well-characterized cocktail of *L. monocytogenes* strains (different serotypes and PFGE types from meat outbreaks) will be surface inoculated and tested in a pilot-plant level.

## **CONCLUSIONS**

Current research demonstrates enhanced inhibitory effect of organic acid salts and natural plant extracts combination when compared to organic acid salts alone (commercial usage level). Furthermore, application of thermal treatment in addition to the antimicrobial agents combination provides enhanced antilisterial effects in the meat products as an alternative approach in processing conditions. This novel approach will be attractive to consumers in addition to providing food safety and health benefits.

## REFERENCES

- Ahn, J., Grun, I.U. and Mustapha, A. 2004. Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. *J Food Prot*, 67, 48–55.
- Apostolidis, E., Kwon, Y.I., Shetty, K., 2008. Inhibition of *Listeria monocytogenes* by oregano, cranberry and sodium lactate combination in broth and cooked ground beef systems and likely mode of action through proline metabolism. *Int J Food Microbiol*, 128, 317–324.
- Bedie, G.K., Samelis, J., Sofos, J.N., Belk, K.E., Scanga, J.A. and Smith, G.C. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4 degrees C in vacuum packages. *J Food Prot*, 64, 1949–1955.
- Bisha, B., Weinsetel, N., Brehm-Stecher, B. F. and Mendonca, A. 2010. Antilisterial effects of gravinol-S grape seed extract at low levels in aqueous media and its potential application as a produce wash. *J Food Prot*, 73, 266–273.
- Cygnarowicz-Provost, M., Whiting, R.C. and Craig, J.C. 1994. Steam surface pasteurization of beef frankfurters. *J Food Sci*, 59, 1–5.
- Doyle, M.E., Mazzotta, A.S., Wang, T., Wiseman, D.W. and Scott, V.N. 2001. Heat resistance of *Listeria monocytogenes*. *J Food Prot*, 64, 410–429.
- Food Safety and Inspection Service (FSIS). (2000). FSIS to increase permissible levels of food ingredients used as antimicrobials and flavoring agents. *Federal Register*, 65, 3121–3123.
- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C.M. 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *J Food Sci*, 73, 389–394.
- Gande, N. and Muriana, P. 2003. Prepackage surface pasteurization of ready-to-eat meats with a radiant oven for reduction of *Listeria monocytogenes*. *J Food Prot*, 66, 1623–1630.
- Glass, K.A. and Doyle, M.P. 1989. Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Appl Environ Microbiol*, 55, 1565–1569.
- Grau, F.H. and Vanderlinde, P.B. 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. *J Food Prot*, 55,4–7.
- Hansen, T. B. and Knochel, S. 2001. Factors influencing resuscitation and growth of heat injured *Listeria monocytogenes* in sous vide cooked beef. *Int J Food Microbiol*, 63, 135–147.
- Hunter, D.R. and Segal, I.H. 1973. Effect of weak acids on amino acid transport by *Penicillium chrysogenum* evidence for proton or charge gradient as the driving force. *J Bacteriol*, 113, 1184–1192.

- Juncher, D., Vestergaard, C.S., Soltoft-Jensen, J., Weber, C.J., Bertelsen, G. and Skibsted, L.H. 2000. Effects of chemical hurdles on microbiological and oxidative stability of a cooked cured emulsion type meat product. *Meat Sci*, 55, 483–491.
- Juneja, V.K. and Eblen, B. 1999. Predictive thermal inactivation model for *Listeria monocytogenes* with temperature, pH, NaCl, and sodium pyrophosphate as controlling factors. *J Food Prot*, 62, 986–993.
- Juneja, V.K., Eblen, B.S., and Marks, H.M. 2001. Modeling non-linear survival curves to calculate thermal inactivation of *Salmonella* in poultry of different fat levels. *Int J Food Microbiol*, 70, 37–51.
- Juneja, V.K., Eblen, B.S. and Ransom, G.M. 2002. Thermal inactivation of *Salmonella* spp. in chicken broth, beef, pork, turkey, and chicken; determination of D- and Z values. *J Food Sci*, 66, 146–152.
- Juneja, V.K., Hwang, C.A. and Friedman, M. 2010. Thermal inactivation and post-thermal treatment growth during storage of multiple *Salmonella* serotypes in ground beef as affected by sodium lactate and oregano oil. *J Food Sci*, 75, M1–M6.
- Kajiya, K., Hojo, H., Suzuki, M., Nanjo, F., Kumazawa, S. and Nakayama, T. 2004. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *J Agri Food Chem*, 52, 1514–1519.
- Knight, T.D., Castillo, A., Maxim, J., Keeton, J.T. and Miller, R.K. 2007. Effectiveness of potassium lactate and sodium diacetate in combination with irradiation to control *Listeria monocytogenes* on frankfurters. *J Food Sci*, 72, 26–30.
- Lau, D.W. and King, A.J. 2003. Pre and post-mortem use of grape seed extract in dark poultry meat to inhibit development of thiobarbituric acid reactive substances. *J Agri Food Chem*, 51, 1602-1607.
- Leistner, L. 2000. Basic aspects of food preservation by hurdle technology. *Int J Food Microbiol*, 55, 181–186. Leo, A., Hansch, C. & Elkins, D. (1971). Partition coefficients and their uses. *Chem Reviews*, 71, 525- 631.
- Lin, Y.T., Kwon, Y.I., Labbe, R.G. and Shetty, K., 2005. Inhibition of *Helicobacter pylori* and associated urease by oregano and cranberry phytochemical synergies. *Appl Environ Microbiol*, 71, 8558–8564.
- Lungu, B. and Johnson, M. G. 2005a. Fate of *Listeria monocytogenes* inoculated onto the surface of model turkey frankfurter pieces treated with zein coatings containing nisin, sodium diacetate, and sodium lactate at 4 °C. *J Food Prot*, 68, 855–859.
- Mackey, B.M., Pritchett, C., Norris, A. and Mead, G.C. 1990. Heat resistance of *Listeria*: strain differences and effects of meat type and curing salts. *Lett Appl Microbiol*, 10, 251–255.

- Mbandi, E. and Shelef, L.A. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int J Food Microbiol*, 76, 191-198.
- McCormick, K.E., Han, I.Y., Acton, J.C., Sheldon, B.W. and Dawson, P.L. 2005. In-package pasteurization combined with biocide impregnated films to inhibit *Listeria monocytogenes* and *Salmonella* Typhimurium in turkey bologna. *J Food Sci*, 70, 52-57.
- Miller, R.K. and Acuff, G.R. 1994. Sodium lactate affects pathogens in cooked beef. *J Food Sci* 59, 15-19.
- Muriana, P.M., Gande, N., Robertson, W., Jordan, B. and Mitra, S. 2004. Effect of prepackage and postpackage pasteurization on postprocess elimination of *Listeria monocytogenes* on deli turkey products. *J Food Prot*, 67, 2472–2479.
- Murphy, R.Y., Duncan, L.K., Driscoll, K.H., Beard, B.L., Berrang, M.B. and Marcy, J.A. 2003. Determination of thermal lethality of *Listeria monocytogenes* in fully cooked chicken breast fillets and strips during postcook in-package pasteurization. *J Food Prot*, 66, 578–583.
- Murphy, R.Y., Osaili, T., Duncan, L.K. and Marcy, J.A. 2004. Thermal inactivation of *Salmonella* and *Listeria monocytogenes* in ground chicken thigh/leg meat and skin. *Poul Sci*, 83, 1218–1225.
- Murphy, R.Y., Hanson, R.E., Feze, N., Johnson, N.R., Scott, L.L. and Feze, N. 2005. Eradicating *Listeria monocytogenes* from fully cooked franks by using an integrated pasteurization-packaging system. *J Food Prot*, 68, 507-511.
- Murphy, R.Y., Hanson, R.E., Johnson, N.R., Chappa, K. and Berang, M.E. 2006. Combining organic acid treatment with steam pasteurization to eliminate *Listeria monocytogenes* on fully cooked frankfurters. *J Food Prot*, 69, 47–52.
- Nebrink, E., Borch, E., Blom, H. and Nesbakken, T. 1999. A model based on absorbance data on the growth rate of *Listeria monocytogenes* and including the effects of pH, NaCl, Na-lactate and Na-acetate. *Int J Food Microbiol*, 47, 99–109.
- O'Bryan, C.A., Grandall, P.G., Martin, E.M., Griffis, C.L. and Johnson, M.G. 2006. Heat resistance of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Listeria innocua* M1, a potential surrogate for *Listeria monocytogenes* in meat and poultry: A review. *J Food Sci*, 71, 23-30.
- Porto, A.C.S., Call, J.E. and Luchansky, J.B. 2004. Effect of reheating on viability of a five-strain mixture of *Listeria monocytogenes* in vacuum-sealed packages of frankfurters following refrigerated or frozen storage. *J Food Prot*, 67, 71–76.
- Perumalla, A.V.S. and Hettiarachchy, N.S. 2011. Green tea and grape seed extracts – Potential applications in food safety and quality. *Food Res Int*, 44, 827-839.

- Poysky, F.T., Paranjpye, R.N., Peterson, M.E., Pelroy, G.A., Guttman, A.E. and Eklund, M.W. 1997. Inactivation of *Listeria monocytogenes* on hot-smoked salmon by the interaction of heat and smoke or liquid smoke. *J Food Prot*, 60, 649–654.
- Ricke, S.C., Kundinger, M.M., Miller, D.R. and Keeton, J.T. 2005. Alternatives to antibiotics: Chemical and physical antimicrobial interventions and foodborne pathogen response. *Poult Sci*, 84, 667–675.
- Roering, A.M., Wierzba, R.K., Ihnot, A.M. and Luchansky, J.B. 1998. Pasteurization of vacuum-sealed packages of summer sausage inoculated with *Listeria monocytogenes*. *J Food Saf*, 18, 49–56.
- Samelis, J., Bedie, G.K., Sofos, J.N., Belk, K.E., Scanga, J.A. and Smith, G.C. 2002. Control of *Listeria monocytogenes* with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4°C in vacuum packages. *J Food Prot*, 65, 299-307.
- Schultze, K.K., Linton, R., Cousin, M.A., Luchansky, J.B. and Tamplin, M.L. 2006. A predictive model to describe the effects of temperature, sodium lactate, and sodium diacetate on the inactivation of serotype 4b strain of *Listeria monocytogenes* in frankfurter slurry. *J Food Prot*, 69, 1552–1560.
- Seman, J.L., Borger, A.C., Meyer, J.D., Hall, P.A. and Milkowski, A.L. 2002. Modeling of the growth of *Listeria monocytogenes* in cured ready-to-eat meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. *J Food Prot*, 65, 651–658.
- Sivarrooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2007. Inhibition of *Listeria monocytogenes* using nisin with grape seed extract on turkey frankfurters stored at 4 and 10 °C. *J Food Prot*, 70, 1017–1020.
- Sivarrooban, T., Hettiarachchy, N.S. and Johnson, M.G. 2008. Transmission electron microscopy study of *Listeria monocytogenes* treated with nisin in combination with either grape seed or green tea extracts. *J Food Prot*, 71, 2105–2109.
- Sommers, C.H., Fan, X., Niemira, B.A. and Sokorai, K. 2003. Radiation (gamma) resistance and post-irradiation growth of *Listeria monocytogenes* suspended in beef bologna that contained sodium diacetate and potassium lactate. *J Food Prot*, 66, 2051–2056.
- Sommers, C.H., Geveke, D.J., and Fan, X. 2008. Inactivation of *Listeria innocua* on frankfurters that contain potassium lactate and sodium diacetate by flash pasteurization. *J Food Sci*, 73, 72 – 74.
- Smith, S.E., Maurer, J.L., Orta-Ramirez, A., Ryser, E.T. and Smith D.M. 2001. Thermal inactivation of *Salmonella spp.*, *Salmonella Typhimurium*, and *Escherichia coli* O157:H7 in ground beef. *J Food Sci*, 66, 1164–1168.

Theivendran, S., Hettiarachchy, N.S. and Johnson, M.G. 2006. Inhibition of *Listeria monocytogenes* by nisin combined with grape seed extract or green tea extract in soy protein film coated on turkey frankfurters. *J Food Sci*, 71, 39–44.

U.S. Department of Agriculture, Food Safety and Inspection Service. 2003. Control of *Listeria monocytogenes* in ready-to-eat meat and poultry products; final rule, 9 CFR Part 430: 6 June 2003. *Federal Register*, 68, 34208-34254.

Vigil, A., Palou, L.M.E. and Alzamora, S.M. 2005. Naturally occurring compounds - plant sources, pp. 429–451. In P. M. Davidson, J. N. Sofos, and A. L. Branen (ed.), *Antimicrobials in foods*. Marcel Dekker, New York.

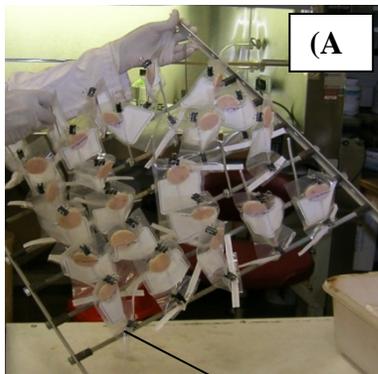
Wederquist, H.J., Sofos, J.N. and schmidt. G.R. 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. *J Food Sci*, 59, 498–500.

Zhu. M., Du, M., Cordray, J. and Ahn, D.U. 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Com Rev Food Sci Food Saf*, 4, 34-42.

**Table 5.1** Water activity and pH determined for low and high fat chicken and turkey hotdog samples.

Treatments	(% weight in formulation)				pH				Water Activity (a <sub>w</sub> )			
	P L	SD	GT E	GS E	Chicken		Turkey		Chicken		Turkey	
					Low	High	Low	High	Low	High	Low	High
<b>Control</b>	0. 0	0.0	0.0	0.0	6.33±0.0 2	6.57±0.0 7	6.36±0.0 3	6.25±0.0 4	0.960±0. 02	0.957±0.0 2	0.967±0.0 1	0.961±0.01
<b>Chemical preservatives</b>	2. 0	0.15	0.0	0.0	6.12±0.0 1	6.30±0.0 1	6.20±0.0 1	6.24±0.0 1	0.963±0. 02	0.960±0.0 1	0.962±0.0 1	0.963±0.02
<b>Chemical preservatives + Plant extracts</b>	1. 5	0.11	0.35	0.22	6.07±0.0 5	6.56±0.0 2	6.17±0.0 1	6.21±0.0 2	0.962±0. 01	0.964±0.0 2	0.958±0.0 2	0.974±0.01

Means are the average of three replications.



(A)



(B)



(C)

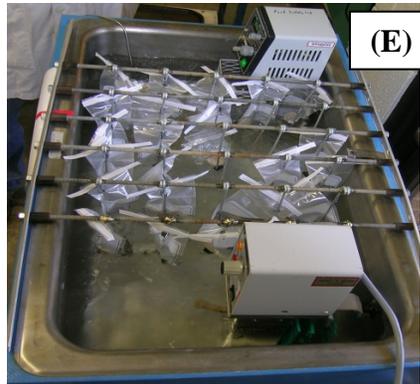
Wire-rack

Vacuum-packed Hotdog

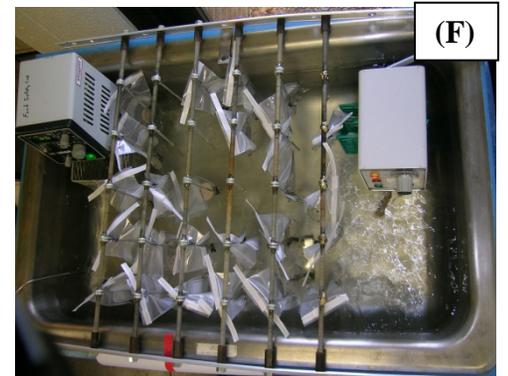
Hot water circulator



(D)

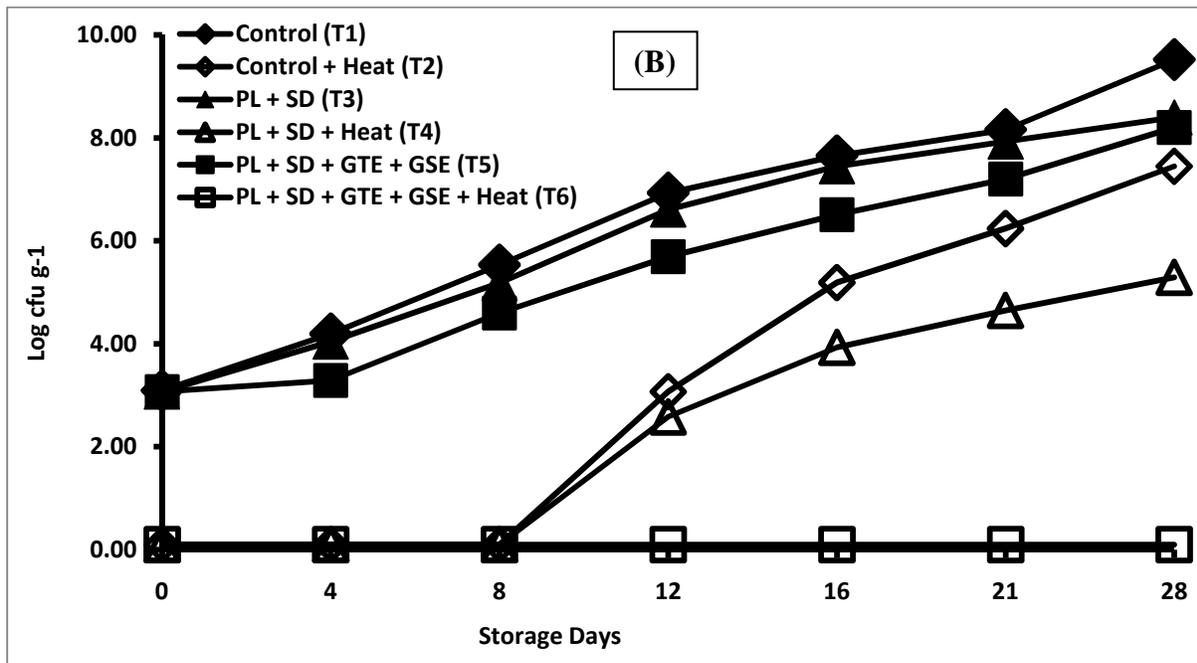
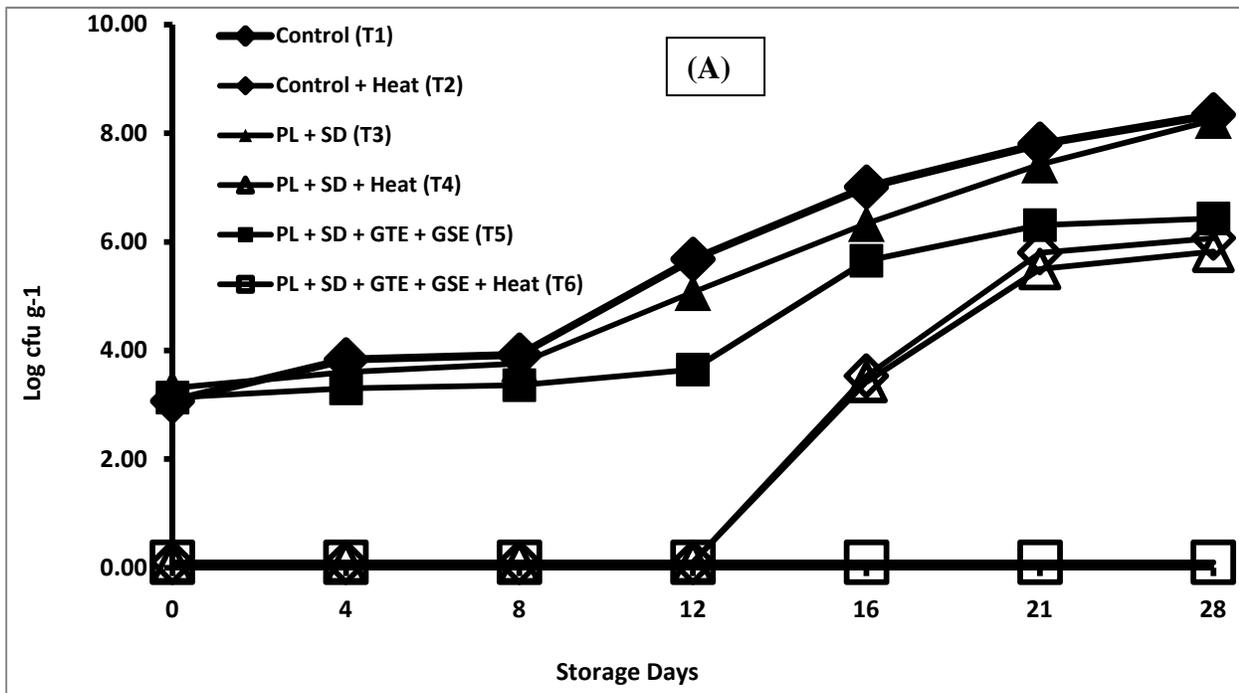


(E)



(F)

**Figure 5.1** Post-packaging thermal treatment of vacuum-packed bags of hotdog samples used in the study. Vacuum-pack bags attached to the wire-rack to support and for immersion in the hot water bath (A, B, C). Hotdog samples subjected to heat treatment in water bath shown at different views (D, E, F).

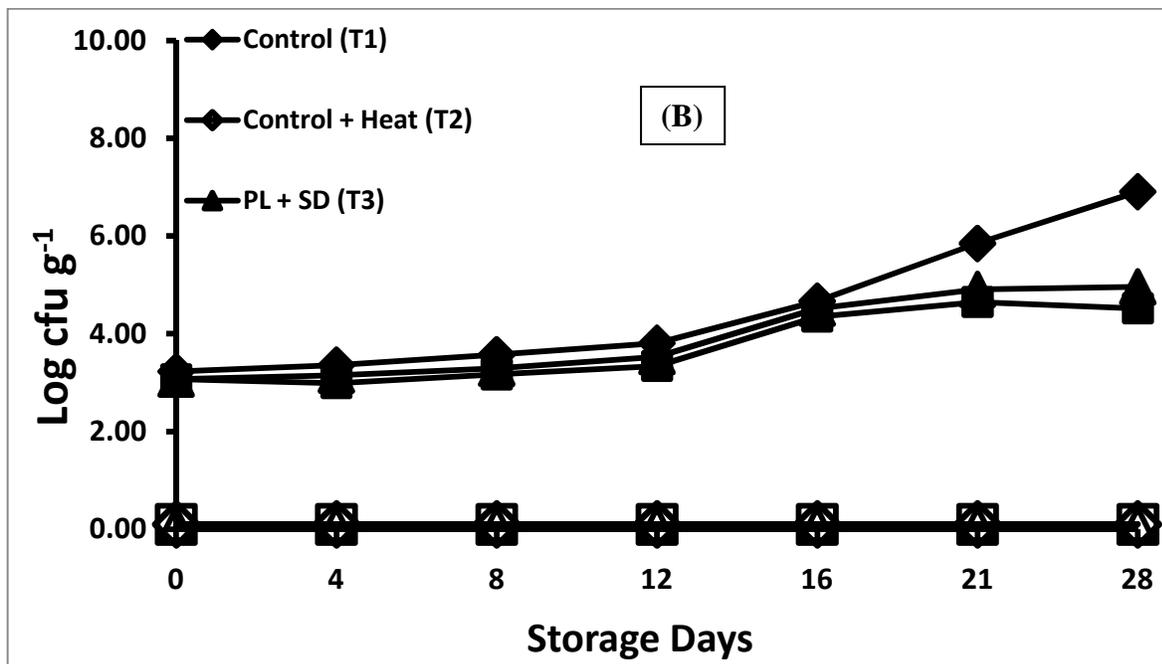
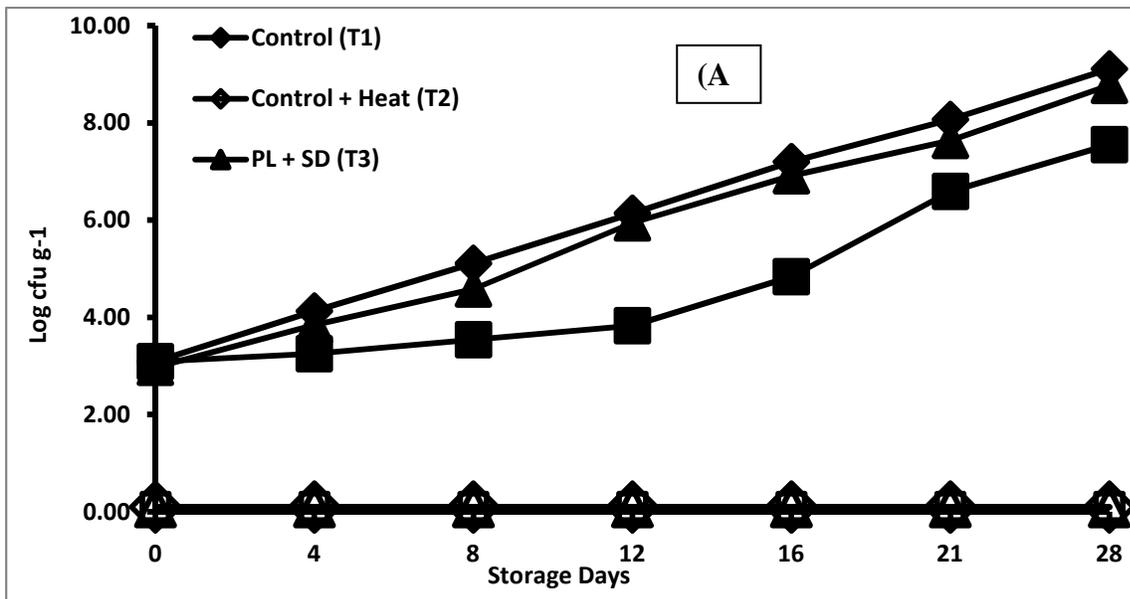


**Figure 5.2 (A & B).** Effect of partial replacement of potassium lactate and sodium diacetate by natural green tea and grape seed extracts and thermal inactivation to inhibit the growth of *Listeria monocytogenes* in low (A) and high fat (B) chicken hotdogs.

Data are mean log numbers ( $\text{cfu g}^{-1}$ ) from three replications observed on 0, 4, 8, 12, 16, 21, and 28 days of storage. Minimum level of detection is  $100 \text{ cfu g}^{-1}$ .

LSD to compare means at same meat or fat or PL- SD- GTE-GSE combinations = 0.31

LSD to compare means at different meat or fat or PL- SD- GTE-GSE combinations = 0.22



**Figure 5.2 (C & D).** Effect of partial replacement of potassium lactate and sodium diacetate by natural green tea and grape seed extracts and thermal inactivation to inhibit the growth of *Listeria monocytogenes* in low (A) and high fat (B) turkey hotdogs.

Data are mean log numbers (cfu g<sup>-1</sup>) from three replications observed on 0, 4, 8, 12, 16, 21, and 28 days of storage. Minimum level of detection is 100 cfu g<sup>-1</sup>.

LSD to compare means at same meat or fat or PL- SD- GTE-GSE combinations = 0.31

LSD to compare means at different meat or fat or PL- SD- GTE-GSE combinations = 0.22.

## CHAPTER VI

### **EFFECT OF PARTIAL REPLACEMENT OF POTASSIUM LACTATE AND SODIUM DIACETATE BY NATURAL GREEN TEA AND GRAPE SEED EXTRACTS ON PHYSICOCHEMICAL AND SENSORY PROPERTIES OF HOTDOGS**

#### **ABSTRACT**

In recent years, using natural food preservatives to replace chemical ingredients has become an increasing trend in the food industry. In this study, green tea extract (GTE) and grape seed extract (GSE) were incorporated in low and high fat chicken and turkey hotdog formulations by partially replacing chemical preservatives such as potassium lactate (PL) and sodium diacetate (SD). Physicochemical and sensory attributes of hotdogs over refrigerated storage (6 weeks) were evaluated in low and high fat chicken and turkey hotdogs. Treatments included were control (no preservatives), potassium lactate (PL), and sodium diacetate (SD) at commercial usage levels (2.0 % and 0.15 % respectively), and partially replaced PL (1.5 %) and SD (0.11 %) by GTE (0.35 %) and GSE (0.22 %) extracts. Addition of GTE and GSE had no significant effect ( $P > 0.05$ ) on water activity and pH values in all meat and fat type hotdogs. Furthermore, GTE and GSE combination inhibited lipid oxidation (no detectable levels of hexanal) until 6<sup>th</sup> week of storage (4 °C) while the hotdog samples formulated with PL and SD oxidized (hexanal) from the 2<sup>nd</sup> week of storage. Consumer panelists observed no significant differences ( $P > 0.05$ ) in all sensory attributes (overall impression, appearance, color, and juiciness) except texture ( $P < 0.05$ ). Consumer panelists ranked higher scores in texture for the hotdogs formulated with chemical preservatives and plant extracts demonstrating that incorporation of GTE and GSE would improve consumer acceptability.

## INTRODUCTION

Ready-to-eat meat (RTE) products such as hotdogs represent a popular segment in convenience food purchases as indicated by its market worth of more than \$1.6 billion in the U.S. supermarkets in the year 2010 (National Hotdog and Sausage Council, 2011). Increased demand has led the processors to extend the shelf life by minimizing foodborne pathogen contamination and lipid oxidation. Post-processing contamination of hotdogs with foodborne pathogens implicating foodborne illness and deaths is a major concern for the processors as well as consumers. Meat lipids undergo auto-oxidation mediated through free-radical reactions that eventually affect the physicochemical and sensory attributes of the product (Brewer, 2009). Therefore, processors are continuously challenged to revisit their strategies to incorporate preservatives (antimicrobials and antioxidants) in hotdog formulations. In general, processors use chemical preservatives such as lactates and diacetates of sodium or potassium salts (antimicrobials) and butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (antioxidants) in commercial hotdog formulations to improve the overall quality and thus extend shelf life. However, use of lactates and diacetates salts at higher levels (above 3.0 % and 0.15 % respectively) have a negative impact on the sensory attributes such as astringent and bitter tastes (Nunez et al., 2004). Use of synthetic antioxidants is limited in meat formulations as consumers are increasingly concerned about their safety, thus encouraged to investigate the natural alternatives (Ahn et al., 2002).

There is a growing interest in food processors and consumers regarding the use of 'natural alternatives' in place of synthetic food additives to prevent the growth of foodborne pathogens and (or) minimizing lipid oxidation (Kulkarni et al., 2011). In recent years, green tea (GTE) and grape seed extracts (GSE) are now being looked to as a better alternative having

demonstrated antioxidant as well as antimicrobial properties in various food applications (Perumalla and Hettiarachchy, 2011). Green tea extract has become popular worldwide in modern times as a promising food additive contributing various health benefits and food applications (Lavelli et al., 2010). The main functional constituents in GTE are catechins, namely (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin gallate (EGCG) (Kajiya et al., 2004). Grape seed extract is also an excellent source of phenolic-rich compounds such as gallic acid, catechin and epicatechin, and procyanidins (Ignat et al., 2010). Polyphenolic compounds present in GTE and GSE have demonstrated potential antioxidant properties due to their redox potential that enable them to act as hydrogen donors, reducing agents, nascent oxygen quenchers, and chelating metal ions in numerous food applications (Ahn et al., 2002; Gramza et al., 2006; Banon et al., 2007; Alghazeer et al., 2008; Brannan, 2009). Furthermore, research findings in our laboratory have demonstrated that partial replacement of potassium lactate (PL) and sodium diacetate (SD) (1.5 % and 0.11 % respectively) by GTE and GSE (0.35 % and 0.22 % respectively) improved growth inhibitions of *Listeria monocytogenes* when compared to hotdogs formulated with lactates and diacetate levels (2.0 % and 0.15 % respectively) at commercial usage level. However, GTE and GSE are colored (light brown and red color respectively) and are rich in catechins, which are known to be astringent and interact with minerals and proteins in addition to antioxidant properties (Perumalla and Hettiarachchy, 2011). Therefore, hotdog formulations including chemical preservatives (organic acids salts – PL and SD) and plant extracts (GTE and GSE) combination may affect physicochemical (pH, water activity, color, and lipid oxidation) and sensory properties (appearance, juiciness, flavor, texture, and overall impression).

This study involving novel application of GSE and GTE combination in poultry hotdog formulations along with PL and SD is expected to satisfy increasing consumer demand for natural products. Our main objective of this study was to evaluate the physicochemical and sensory attributes of hotdogs formulated with PL and SD combinations and green tea and grape seed extracts.

## **MATERIALS AND METHODS**

### **MATERIALS**

Fresh, boneless, skinless chicken breast, mechanically separated chicken (Tyson Foods Inc, Springdale, AR, USA), and mechanically deboned turkey (Cargill Meat Solutions, Springdale, AR, USA) were used to formulate low and high fat chicken and turkey hotdogs. Non-meat ingredients for hotdog preparation include salt, sodium tripolyphosphate, dextrose, monosodium glutamate, (Heartland Supp. Co, AR, USA), red pepper, black pepper (Eatam Foods Company, NJ, USA), sodium nitrite (Southern Indiana Butcher Supply, IN), potassium lactate (PURASAL<sup>®</sup> 60 % HiPure P, Purac America, Lincolnshire, IL), and sodium diacetate (Jarchem, NJ, USA). Non-edible casings were used to stuff the emulsified meat (Casings: 30 mm diameter fibrous cellulose casings; E-Z Peel4 Nojax, 30-84 4STR clear, Viskase Corp., Willowbrook, IL, USA). Natural plant extracts such as GTE (Sunphenon<sup>®</sup> 90M-T, Taiyo International, Inc, Minneapolis, MN) and GSE (MegaNatural<sup>®</sup> Gold Grape Seed Extract, Polyphenolics, Madera, CA) were used in hotdog formulations. Total phenolic contents of the GTE and GSE as determined by Folin Ciocalteu method were 96.3 and 97.3 g gallic acid equivalent per 100 gram of dry material respectively.

### **METHODS**

#### **Hotdog preparation**

Hotdogs were prepared at a poultry processing pilot plant at the University of Arkansas. High fat hotdogs (20 % fat in final product) were formulated using mechanically separated chicken or turkey meat; whereas the low fat (3 % fat in final product) hotdogs were formulated using ground boneless, skinless chicken breast meat or mechanically separated turkey meat. Non-meat ingredients used in the hotdogs preparation include ice, salt, sodium tripolyphosphate, dextrose, sodium nitrite, dextrose, red and black pepper, and monosodium glutamate. In addition, hotdog formulations included chemical preservatives such as potassium lactate (PL), sodium diacetate (SD), and natural plant extracts including GTE and GSE depending on the treatment. Three treatments included were control (no chemical preservatives or plant extracts), chemical preservatives alone (similar to commercial hotdog formulation levels: PL, 2 % and SD, 0.15 %), and partial replacement of chemical preservatives with plant extracts consisting of PL (1.5 %), SD (0.11 %), GTE (0.35 %), and GSE (0.22 %).

Ground meat was mixed with non-meat ingredients and chemical preservatives, plant extract, or both, to form a homogenous emulsion batter in a bowl chopper (Type K64V-VA, Seydelman, Germany). Meat emulsion was transferred to sausage stuffer (Friedrich Dick hand stuffer, 15LTR, Germany) with inedible cellulose casings (30 mm diameter), and slid along the horn of the stuffer. Meat emulsion was stuffed, extruded, pinched and twisted into 6-inch hotdogs links. Hotdogs were placed on cooking sticks in an oven (ALKAR-RapidPak, Inc., Model-1000, Wisconsin, USA) at 82.2 °C until the internal temperature reached 73.8 °C. After cooking, hotdogs were cooled with a shower at 25.5 °C and stored at 4 °C for further studies.

### **Color**

Color determinations were conducted on both external and internal surfaces of longitudinally sliced hotdogs using a chromameter (Minolta colorimeter CR-300, Ramsey, NJ,

USA) over 6 weeks of refrigerated storage at 4 °C. Three hotdogs per treatment at each test week were stripped of the inedible casings, wiped of the drippings, sliced in half longitudinally to open the face of hotdogs, and random readings were taken on the surface and inside of the hotdogs at three different locations. Readings were averaged for surface and internal color separately and expressed as the Hunter L\* (lightness), a\* (redness), and b\* (yellowness) coordinates.

### **pH and water activity analyses**

Mode of action lactate and diacetates is by intracellular acidification and lowering the water activity. Therefore, pH and water activity analyses were determined to investigate the effect storage (4 °C) over time (6 weeks). Hotdogs from each treatment at each test week were homogenized in a food blender (Oster® 16-speed blender; Model-6687, Sunbeam Products, Inc., FL, USA) for pH and water activity ( $a_w$ ) determinations. For pH, 10 grams of homogenized hotdog sample was diluted (1:10 w/v) with distilled water to make a slurry followed by inserting the pH probe (Orion™, Model 720A, Orion research Inc., Beverly, Mass., U.S.A.) to record the readings. Water activity was measured by spreading homogenized sample (~ 5 g) evenly on the sample cup in the water activity meter (Aqua Lab™ model 3 series, Decagon devices Inc., Washington, D.C., USA) at 20 °C. Readings were collected from the samples positioned inside the vapor chamber of the water activity meter after 2 to 3 min. All the analyses were performed in triplicate over the storage period time.

### **Texture analyses**

Firmness of the hotdogs was recorded every week until 6 weeks of refrigerated (4 °C) storage using a texture analyzer (TA.XT2i, Stable Microsystems, UK). Hotdogs removed from refrigerated storage were thawed for 30 min to equilibrate at room temperature. For sample preparation, inedible hotdog casings were removed, wiped off the drip, and portioned (1 cm

length, 12 – 13 mm diameter). A compression probe with a 5-kg load cell was used at 5mm/s pre-test and 10.0 mm/s post-test with a return distance of 25 mm. Hotdog samples were compressed to 70 % of their original height. Peak force (N) of the first compression cycle was recorded and expressed as firmness of the sample.

### **Lipid oxidation - hexanal determination**

Oxidative spoilage of lipids and proteins in meat products such as hotdogs generate compounds such as ketones, alcohols and aldehydes; primarily responsible for warmed-over flavor (WOF) (Estevez and Cava, 2006). Among these, hexanal is mainly generated as a consequence of the oxidative decomposition of poly unsaturated fatty acids (PUFA) in poultry meat and has been used as an indicator of rancid odors associated with lipid oxidation (Brunton et al., 2000; Estevez et al., 2007). For hexanal determination, a GC-MS method was chosen over thiobarbituric acid-reacting substances (TBARS) as the former method is versatile, inexpensive, solvent-free to estimate the volatile compounds (Brunton et al., 2000).

A gas chromatography-mass spectrometry GC-MS (Shimadzu, model Q5050A, Columbia, MD, USA) equipped with a solid-phase microextraction (SPME) sampling system with a 65 µm carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) fiber was used to determine hexanal in hotdogs over refrigerated storage (6 weeks). Hotdog samples (approximately 50 g) from each treatment were homogenized in a food blender (Oster® 16-speed blender; Model-6687, Sunbeam Products, Inc., FL, USA) for 30 s and 7 g was taken for each week of analysis, mixed with 5 mL of saturated NaCl solution, and placed in a 50-mL sample vial with a crimped silicon-septum cap. Prior to GC-MS analysis, the sample vials were incubated at 45 °C for 15 min. At the beginning of the incubation the CAR/PDMS/DVB fiber was inserted 22 mm into headspace above the sample to adsorb volatiles released from the

sample. After incubation, the CAR/PDMS/DVB fiber was desorbed in the injection port of the GC at 250 °C for 5 min. The constituent compounds adsorbed onto the CAR/PDMS/DVB fiber were separated using a Restek XTI-5 column (0.25 mm ID x 30 m x 0.25 µm). The temperature and pressure conditions of the GC were based on optimization work completed prior to this experiment and were 50 °C for 5 min then increased to 200 °C at 20 °C/min. The pressure within the column was held at 47.4 psi for 5 min, and then increased to 7 psi/min to 100 psi. The interface temperature between the GC and MS was held at 280 °C.

### **Consumer test**

Low fat turkey hotdogs were sampled (~ 10 g) and placed in a food warmer (Henny Penny, Model-HCW-3, Eaton, OH, USA) at 60 °C until served, with a maximum holding period of 30 min. A total of 56 panelists between 18 – 55 years of age, from various socio-economic backgrounds and hotdog consumption frequency, were recruited within the university by advertisement (flyer, e-mail, and Facebook) and personal contacts. Consumer sensory testing was conducted at the Department of Food Science Sensory facility, Fayetteville, Arkansas. At the start of the test, panelists were provided with identification cards in the order they appear to the test site. A randomly selected 3-digit numbers were given to the samples with a balance order tasting (Meilgaard et al., 1999). Each consumer was provided a sample tray containing two low fat turkey hotdog samples per treatment. All consumer panelists were provided with unsalted crackers and distilled water at room temperature to cleanse the palette between the samples and also to eliminate carryover factors. A consumer questionnaire was given to the panelists and they were asked to record their scores for overall impression, juiciness, texture, flavor, color, appearance, and preference on a 9-point hedonic scale, with 9 = “like extremely” and 1 = “dislike extremely.” Furthermore, consumers were asked to determine their intensity scores in 5-point

“Just About Right” (JAR) scales, with “just about right” = 3 (1 = “much too weak”; 5 = “much too strong”) for overall flavor, chicken flavor, and juiciness. Store purchase intent was also evaluated on a 5-point scale, with 1 = “definitely would not buy,” 3 = “may or may not buy,” and 5 = “definitely would buy.”

### **Statistical Analysis**

The experiment design was a split plot where the whole plot was completely randomized [two meats (chicken and turkey), X two fats (high and low fat), and X three treatments (including control) and the split plot factor was six weeks of storage (0, 1, 2, 3, 4, 5, 6 weeks) with three replications (three hotdog samples at each sampling time). All data were analyzed by SAS (SAS Inst., Cary, NC) using replicates as random effects and treatments as fixed effects. Mean separations were determined at  $\alpha = 0.05$ . For consumer data, panelists were treated as a random effect and sample treatment was treated as a fixed effect (two samples, one after other, was served and is dependent). The responses to the consumer sensory questions were subjected to one-way analysis of variance and significant differences were determined by least significant difference (LSD) method ( $\alpha = 0.05$ ).

## **RESULTS AND DISCUSSION**

### **Effect on physicochemical attributes**

#### ***Water activity, pH, and color analyses***

Least square means of pH,  $a_w$ , and color (surface and internal) are presented in Table 1. Water activity ( $a_w$ ) was significantly ( $P < 0.05$ ) decreased by the addition of chemical preservatives (PL and SD) and plant extracts (GTE and GSE) combinations in low fat treatments (chicken and turkey) when compared to control and chemical preservatives alone treatments. However, there were no significant differences ( $P > 0.05$ ) in  $a_w$  in high fat hotdogs (chicken and

turkey) among all the treatments. Bedie et al. (2001) reported that addition of lactates has shown no significant effect in reducing  $a_w$  values in frankfurter formulations. These variations may be due to the interaction of water molecules (higher in low fat than high fat treatments) with the water-soluble compounds in GTE and GSE and thus reducing the availability of free water. This may significantly ( $P < 0.05$ ) impact the textural attributes such as firmness as indicated by lower firmness values in low fat samples (chicken and turkey) compared to high fat samples. Furthermore, consumer panelists also preferred turkey low fat samples formulated with chemicals and plant extracts than samples formulated with chemical preservatives alone. No significant differences ( $P > 0.05$ ) in pH were found by the addition of chemical preservatives alone or in combination with plant extracts when compared to control regardless of meat and fat type (Table 1). The pH of 1 % GTE and GSE solutions used in this study were 4.9 and 5.3, respectively. These results were consistent with the previous findings that addition of lactates (Mbandi and Shelef, 2002; Porto et al., 2002) or plant-by products such as grape seed flour and GSE (Ahn et al., 2007; Ozvural et al., 2011) had no significant effect on pH values.

Changes in surface color ( $L^*$ ,  $a^*$ ,  $b^*$ ) were unaffected by including PL and SD in the hotdog formulations (low and high fat chicken and turkey). Addition of chemical preservatives and plant extracts combination did not significantly ( $P > 0.05$ ) change the surface  $L^*$  (Lightness) values across all meat and fat types. However, treatment with chemical preservatives and plant extracts combination significantly ( $P < 0.05$ ) increased surface  $a^*$  (redness) values in low fat chicken and turkey hotdogs when compared to control samples. Although these differences were significant, the color space value differences were of such small magnitude as indicated by no differences ( $P < 0.05$ ) observed and both treatments color were equally preferred by the consumer panelists (Table 4). Addition of chemical preservatives alone had decreased the surface  $b^*$

(yellowness) values and further decreased significantly ( $P < 0.05$ ) when both chemical preservatives and plant extracts were incorporated in the hotdog formulation among all the meat and fat types. Changes in the surface color may possibly be the result of adding GTE and GSE (light brownish and red color extracts, respectively) and thereby suppressing the meats' natural color, and/or changing of emulsion color because of potential variation in the emulsion properties of the each treatment combination (Ozvural et al., 2011). Addition of chemical preservatives, plant extracts, or both, had a small effect on the internal color ( $L^*$ ,  $a^*$ ,  $b^*$ ) values among all meat and fat type hotdogs. Hotdog storage under refrigerated conditions (over 6 weeks) had a minimal effect on pH,  $a_w$ , color (surface and internal) values (Table 2a & 2b).

#### ***Effect on texture***

Means of firmness values of low and high fat chicken and turkey hotdog samples over storage time are presented in Table 2. Firmness values observed a decreasing trend over time (i.e. 6 weeks) in all meat and fat type evidently by oxidation of lipids and proteins and decomposition by spoilage microorganisms. Firmness values were significantly ( $P < 0.05$ ) affected by the addition of chemical preservatives alone and in combination with plant extracts as indicated by higher values in control samples. Hotdog (chicken and turkey) samples formulated with chemical preservatives and plant extracts were significantly lower than samples formulated with chemical preservatives alone. Incorporating GTE and GSE improved the textural attributes such as firmness in all the samples (chicken and turkey) as indicated by significantly ( $P < 0.05$ ) higher preference scores by consumer panelists. Fat level has a significant effect ( $P < 0.05$ ) on firmness values as high fat hotdog samples have lower values (i.e. softer) than low fat samples.

#### ***Effect on lipid oxidation – hexanal determination***

Lipid oxidation in the hotdogs was determined every week until 7 weeks of storage (4 °C) by quantifying hexanal (ppm) as an indicator of meat spoilage. Identification of hexanal was first detected on 2<sup>nd</sup> week (data not shown) in high fat chicken and turkey samples formulated with PL (2.0 %) and SD (0.15 %) and thereby also observed in control samples with increasing hexanal concentration over time until the 7<sup>th</sup> week (Table 3). Oxidation of lipid and protein present in the hotdog samples liberated peroxides (dibutyl) and acetamides as detected from the headspace analyses. By the 6<sup>th</sup> week of storage, all samples except the treatments with GTE and GSE regardless of meat and fat type were spoiled, evidently with the increased hexanal concentrations (Table 3). Hexanal was not detected until 7<sup>th</sup> week of storage in the hotdog samples formulated with partially replaced PL (0.15 %) and SD (0.11 %) with GTE (0.35 %) and GSE (0.22 %). This can be explained by the ability of GTE and GSE to inhibit the formation of conjugated di-ene hydro peroxides and hexanal in the system (Frankel et al., 1994). As expected, high fat samples (chicken and turkey) have higher hexanal values owing to amount of fat susceptible to oxidation process. Hexanal values were found to be significantly ( $P < 0.05$ ) higher in hotdog samples formulated with PL and SD when compared to control as salts may demonstrate prooxidant effect (Rhee et al., 1983). Hexanal values were slightly higher ( $P > 0.05$ ) in turkey samples when compared to their chicken counterparts.

Differences in hexanal content (i.e. lipid oxidation) between meats (chicken and turkey) and fat (low and high) exist due to various factors. Rate of lipid oxidation depends on various factors such as composition of raw meat, deboning process, emulsification process, and additives such as salt, nitrite, spices, and antioxidants (Kanner, 1994; Rhee, 1988). In addition, differences in total lipid content and fatty acid composition, phospholipids, and polyunsaturated fatty acids (PUFA) content in chicken and turkey would ultimately direct the development of lipid

peroxidation in the cooked meat products (Jeun-Horng et al., 2002). Another factor in consideration for variation would be the complex interfacial phenomena involved as the lipophilicity, solubility, and partition between aqueous and lipid phase may become important in determining antioxidant activity (Frankel et al., 1994).

### **Effect on sensory attributes**

#### ***Consumer test***

Means for the consumer test attributes recorded on hedonic scale (9-point) and “Just about right” (JAR – 5 point) were summarized in Table 4a and 4b. Low fat turkey hotdog samples formulated with commercial usage level of PL (2.0 %) and SD (0.15 %) were considered as the “control” treatment, while “Test” samples include low fat turkey hotdogs formulated with chemical preservatives (1.5 % PL and 0.15 % SD) partially replaced by natural plant extracts (0.35 % GTE and 0.22 % GSE). “Overall impression” is an integrated product descriptor used to evaluate the balance and blend of the products attributes such as color, flavor, texture, and juiciness (Lawless and Heyman, 1984). Partial replacement of chemical preservatives by natural green tea and grape seed extracts had no significant effect ( $P > 0.05$ ) on overall impression of the hotdogs compared to the treatments formulated with chemical preservatives alone. In addition, results for the sensory attributes such as appearance, color, flavor, and juiciness were similar to those of overall impression; no significant differences ( $P > 0.05$ ) between the control and test product samples (Table 4a). However, test product was categorically ranked as higher in appearance, flavor, juiciness, texture, and overall impression. Significant differences in texture were ( $P < 0.05$ ) observed by consumer panelists with test samples having higher values i.e. high acceptability than control samples. Similar results were reported by Nunez et al. (2004) that incorporation of PL had no significant effect on textural attributes when compared to control

(without PL) in beef frankfurters. Both the treatments were categorized as “neither like nor dislike” to “like slightly” with test samples ranking higher based on the 9-point hedonic scales (Table 4a). These results were consistent with the findings by Ozvural et al. (2011) that grape seed flour (0.5 %) can be incorporated into frankfurter formulations without affecting sensory attributes. Rababah et al. (2005) reported that chicken breasts infused with green tea (0.3 %) and grape seed extract (0.3 %) combinations had no significant effect ( $P < 0.05$ ) on overall acceptance of flavor, texture, and color. These results suggest that that green tea (0.35 %) and grape seed extracts (0.22 %) can be used in the ready-to-eat meat product formulations without impacting the sensory attributes while providing additional health benefits.

Frequency distributions (%) for the attributes including overall impression, appearance, color, flavor, texture, and juiciness are presented in Table 6 for the hedonic scale. Consumer panelists ranked higher scores (61.54 %) for test samples than control (51.94 %) for “overall impression” suggesting incorporating GTE and GSE may improve the consumer acceptability. With regard to texture, a greater percentage (69.23) of consumer panelists liked (“like slightly” to “like very much”) the test product, whereas a greater percentage (53.85) of consumers disliked (“dislike slightly” to dislike extremely”) the control product. Although not significantly different, a greater percentage of the consumers liked (“like slightly” to “like very much”) juiciness of test product (61.53 %) compared to control (57.7 %). Consumer responses were also asked to record the intensity of the attributes including color, firmness, overall flavor, juiciness, and store purchase intent on “just about right” scales (JAR). Frequency distributions (%) for the JAR scale attributes including color, firmness, overall flavor, juiciness, and purchase intent are presented in Table 7. This scale involves the ranking of the attribute on 5-point level (bipolar in nature) with extreme ends indicating opposite sensory descriptors and the middle category indicating “just

about right” as most acceptable (Gacula et al., 2006). The JAR for color indicated that approximately 80 % of the panelists categorized as “much too light” to “too light” in both the treatments, suggesting that GTE and GSE blended with turkey meat color and had no effect on the color of final product as perceived by the consumer panelists. Similar results were observed in overall flavor, firmness, and juiciness, where the JAR scales were not affected by the chemical preservatives alone and in combination with plant extracts. Panelists were also asked for their intent as a potential store purchase by providing the required information regarding natural plant extract and their benefits. Purchase price was not included in this assessment. There was no significant difference ( $P > 0.05$ ) in purchase intent control and test product (Table 7). These results indicate that partial replacement of conventional chemical preservatives (PL and SD) by natural plant extracts (GTE and GSE) would be beneficial to RTE meat processors without impacting the sensory attributes of the hotdog samples.

## CONCLUSIONS

This study shows that both GTE and GSE were effective in poultry hotdog formulations and can partially replace conventional chemical preservatives such as PL and SD without affecting physicochemical and sensory attributes. Furthermore, GTE and GSE demonstrated antioxidant properties by extending the shelf life of hotdogs until 6 weeks of storage at 4 °C. Although there are no adverse effect of plant extracts on color, flavor, texture, and juiciness, application of these extracts at higher levels might be limited due to the possible interaction of these extracts with minerals and protein and astringent properties of phenolic rich compounds.

## REFERENCES

- Ahn, J., Grun, I.U. and Fernando, L.N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *J Food Sci*, 67, 1364–1369.
- Ahn, J., Grün, I.U. and Mustapha, A. 2007. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiol*, 24, 7–14.
- Alghazeer, R., Saeed, S., and Howell, N.K. 2008. Aldehyde formation in frozen mackerel (*Scomber scombrus*) in the presence and absence of instant green tea. *Food Chem*, 108, 801–810.
- Banon, S., Diaz, P., Rodriguez, M., Garrido, M.D. and Price, A. 2007. Ascorbate, green tea and grape seed extracts increase the shelf life of low sulphite beef patties. *Meat Sci*, 77, 626–633.
- Bedie, G.K., Samelis, J., Sofos, J.N., Belk, K.E., Scanga, J.A., and Smith, G.C. 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* postprocessing contamination on frankfurters stored at 4 °C in vacuum packages. *J Food Prot*, 64, 1949–1955.
- Bozkurt, H. 2006. Utilization of natural antioxidants: Green tea extract and *Thymbra spicata* oil in turkish dry-fermented sausage. *Meat Sci*, 73, 442–450.
- Bradford, D.D., Huffman, D.L., Egbert, W.R. and Mikel, W.B. 1993. Potassium lactate effects on low fat fresh pork sausage chubs during simulated retail distribution. *J Food Sci*, 58, 1245–1248.
- Brewer, M.S. 2009. Irradiation effects on meat flavor: A review. *Meat Sci*, 81, 1-14.
- Brannan, R.G. 2009. Effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage. *Meat Sci*, 81, 589–595.
- Brunton, N.P. Cronin, D.A., Monahan, F.J. and Durcan, R.A. 2000. Comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food Chem*, 68, 339-345.
- Estevez, M. and Cava, R. 2006. Effectiveness of rosemary essential oil as an inhibitor of lipid and protein oxidation: contradictory effects in different types of frankfurters. *Meat Sci*, 72, 348–355.
- Estévez, M., Ventanas, S. and Cava, R. 2007. Oxidation of lipids and proteins in frankfurters with different fatty acid compositions and tocopherol and phenolic contents. *Food Chem*, 100, 55–63.
- Frankel, E.N., Huang, S.W., Kanner, J. and German, J.B. 1994. Interfacial Phenomena in the evaluation of Antioxidants: Bulk Oil vs. Emulsions, *J Agric Food Chem*, 42, 1054–1059.

- Gacula, M., Rutenbeck, S., Pollack, L., Resurreccion, A.V.A. and Moskowitz, H. 2006. The just-about-right intensity scale: Functional analysis and relation to hedonics. *J Sens Stud*, 22, 194–211.
- Gramza, A., Khokhar, S., Yoko, S., Gliszczynska-Swiglo, A., Hes, M. and Korczak, J. 2006. Antioxidant activity of tea extracts in lipids and correlation with polyphenol content. *Eur J Lipid Sci Technol*, 108, 351–362.
- Ignat, I., Stingu, A., Volf, I., and Popa, V.I. 2011. Characterization of grape seeds aqueous extract and possible application in biological system. *Cellul Chem Technol*, 45, 205–209.
- Jeun-Horng, L., Yuan-Hui, L., and Chun-Chin, K. 2002. Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Sci*, 60, 161–167.
- Kajiya, K., Hojo, H., Suzuki, M., Nanjo, F., Kumazawa, S., and Nakayama, T. 2004. Relationship between antibacterial activity of (+)-catechin derivatives and their interaction with a model membrane. *J Agri Food chem*, 52, 1514–1519.
- Kanner J. 1994. Oxidative processes in meat and meat products: Quality implications. *Meat Sci*, 36, 169-189.
- Kulkarni, S., Desantos, F.A., Kattamuri, S., Rossi, S.J. and Brewer, M.S. 2011. Effect of grape seed extract on oxidative, color and sensory stability of a pre-cooked, frozen, re-heated beef sausage model system. *Meat Sci*, 88, 139–144.
- Lavelli, V., Vantaggi, C., Corey, M. and Kerr, W. 2010. Formulation of a dry green tea apple product: Study on antioxidant and color stability. *J Food Sci*, 75, 184–190.
- Lawless, H.T., and Heymann, H. 1984. Descriptive analysis. pp: 347–348 in *Sensory Evaluation of Food: Principles and Practices*. Chapman and Hall, Int. Thomson Publ., Florence, KY.
- National Hot and Sausage Council. 2011. Vital hotdog statistics. Accessed at <http://www.hot-dog.org/ht/d/sp/i/38579/pid/38579>, December 2011.
- Mason, T.L. and Wasserman, B.P. 1987. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. *Phytochem*, 26, 2197–2202.
- Mbandi, E., and Shelef, L.A. 2002. Enhanced antimicrobial effects of combination of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. *Int J Food Microbiol*, 76, 191–198.
- Meilgaard, M. Civille, G.V. and Carr, B.T. 1999. *Sensory Evaluation Techniques*. 3rd ed. CRC Press Inc., Boca Raton, FL.

Nuñez De Gonzalez, M.T., Keeton, J.T. and Ringer, L.J. 2004b. Sensory and physicochemical characteristics of frankfurters containing lactate with antimicrobial surface treatments. *J Food Sci*, 69, 221–228.

Ozvural, E.B. and Vural, H. 2011. Grape seed flour is a viable ingredient to improve the nutritional profile and reduce lipid oxidation of frankfurters. *Meat Sci*, 88, 179-183.

Perumalla, A.V.S. and Hettiarachchy, N.S. 2011. Green tea and grape seed extracts–Potential applications in food safety and quality. *Food Res Int*, 44, 827-839.

Rababah, T., Hettiarachchy, N.S., Eswaranandam, S., Meullenet, J.F., and Davis, B. 2005. Sensory evaluation of irradiated and nonirradiated poultry breast meat infused with plant extracts. *J Food Sci*, 70, 228–235.

Rhee, K.S., Smith, G.C, and Terrell, R.N. 1983. Effect of reduction and replacement of sodium chloride on rancidity development in raw and cooked ground pork. *J Food Prot*, 46, 578-581.

Rhee, K.S. 1984. Enzymic and nonenzymic catalysis of lipid peroxidation in muscle foods. *Food Technol*, 42, 127-132.

**Table 1** Least square means of pH,  $a_w$ , surface, and internal color values of low and high fat chicken and turkey hotdogs averaged across 6 weeks of refrigerated storage

Meat	Fat level	Treatment	$a_w$	pH	Surface color			Internal color		
					$L^*$	$a^*$	$b^*$	$L^*$	$a^*$	$b^*$
Chicken	Low (3 %)	Control (T1)	0.968	6.20	95.49	0.28	2.20	94.78	0.39	0.87
		PL + SD (T2)	0.963	6.03	96.76	0.28	2.53	93.46	1.06	1.38
		PI + SD + GTE + GSE (T3)	0.957	6.10	94.69	1.30	0.64	90.76	2.77	0.72
	High (20 %)	Control (T1)	0.959	6.50	88.62	3.29	3.67	90.96	3.48	2.36
		PL + SD (T2)	0.962	6.40	91.23	1.61	4.08	91.88	2.55	2.50
		PI + SD + GTE + GSE (T3)	0.966	6.54	89.85	1.19	2.75	89.84	1.27	1.60
Turkey	Low (3 %)	Control (T1)	0.969	6.24	90.91	1.49	2.40	91.98	3.40	2.07
		PL + SD (T2)	0.967	6.30	91.19	2.07	2.73	89.34	3.54	3.45
		PI + SD + GTE + GSE (T3)	0.960	6.25	90.48	2.72	1.00	88.81	1.90	2.82
	High (20 %)	Control (T1)	0.963	6.35	90.60	2.86	2.50	90.51	7.56	0.18
		PL + SD (T2)	0.959	6.39	90.71	2.46	3.73	90.91	6.49	0.12
		PI + SD + GTE + GSE (T3)	0.965	6.34	88.17	2.48	2.48	89.23	6.48	0.88

T1: No chemical preservatives or plant extracts, T2: PL (2.0 %) + SD (0.15 %), T3: PL (0.15 %) + SD (0.11 %) + GTE (0.35 %) + GSE (0.22 %).

LSD to compare means in pH at same or different meat or fat or PL- SD- GTE-GSE combinations for - 0.32

LSD to compare means in  $a_w$  at same or different meat or fat or PL- SD- GTE-GSE combinations - 0.11

LSD to compare means in internal color ( $L^*$   $a^*$   $b^*$ ) at same meat and fat and PL- SD- GTE-GSE combinations = 1.98 ( $L^*$ ), 1.43 ( $a^*$ ), 1.43 ( $b^*$ )

LSD to compare means in internal color ( $L^*$   $a^*$   $b^*$ ) at different meat or fat or PL- SD- GTE-GSE combinations = 2.01( $L^*$ ), 1.45 ( $a^*$ ), 1.44( $b^*$ )

LSD to compare means in surface color ( $L^*$   $a^*$   $b^*$ ) at same meat and fat and PL- SD- GTE-GSE combinations = 2.0( $L^*$ ), 0.8( $a^*$ ), 1.1( $b^*$ )

LSD to compare means in surface color ( $L^*$   $a^*$   $b^*$ ) at different meat or fat or PL- SD- GTE-GSE combinations = 1.9( $L^*$ ), 0.8( $a^*$ ), 1.15( $b^*$ )

**Table 2** Means of firmness (N) values of low and high fat turkey hotdogs formulated with chemical preservatives and/or plant extracts averaged across 6 weeks of refrigerated storage

Meat	Fat level	Treatment	Storage (weeks)						
			0	1	2	3	4	5	6
Chicken	Low (3 %)	Control	393.4 <sup>a</sup>	287.3 <sup>a</sup>	329.2 <sup>a</sup>	281.9 <sup>a</sup>	257.0 <sup>a</sup>	181.4 <sup>a</sup>	160.6 <sup>a</sup>
		PL + SD	322.5 <sup>b</sup>	270.3 <sup>a</sup>	273.8 <sup>b</sup>	241.7 <sup>b</sup>	206.0 <sup>b</sup>	170.6 <sup>a</sup>	139.1 <sup>a</sup>
		PI + SD + GTE + GSE	199.3 <sup>c</sup>	194.6 <sup>b</sup>	135.3 <sup>c</sup>	222.0 <sup>b</sup>	197.0 <sup>b</sup>	140.3 <sup>a</sup>	135.7 <sup>a</sup>
	High (20 %)	Control	125.8 <sup>a</sup>	111.2 <sup>a</sup>	92.0 <sup>a</sup>	112.5 <sup>a</sup>	157.7 <sup>a</sup>	121.3 <sup>a</sup>	119.7 <sup>a</sup>
		PL + SD	91.1 <sup>b</sup>	83.9 <sup>b</sup>	70.3 <sup>b</sup>	99.3 <sup>a</sup>	82.7 <sup>b</sup>	56.6 <sup>b</sup>	47.6 <sup>b</sup>
		PI + SD + GTE + GSE	68.5 <sup>c</sup>	51.8 <sup>c</sup>	54.3 <sup>b</sup>	62.4 <sup>b</sup>	56.2 <sup>b</sup>	47.1 <sup>b</sup>	46.3 <sup>b</sup>
Turkey	Low (3 %)	Control	251.6 <sup>a</sup>	218.4 <sup>a</sup>	242.1 <sup>a</sup>	240.9 <sup>a</sup>	160.6 <sup>a</sup>	169.0 <sup>a</sup>	143.1 <sup>a</sup>
		PL + SD	205.5 <sup>b</sup>	202.5 <sup>a</sup>	167.6 <sup>b</sup>	207.2 <sup>b</sup>	139.1 <sup>a</sup>	163.3 <sup>a</sup>	127.8 <sup>a</sup>
		PI + SD + GTE + GSE	120.2 <sup>c</sup>	100.0 <sup>b</sup>	103.9 <sup>c</sup>	106.3 <sup>c</sup>	135.7 <sup>a</sup>	92.3 <sup>b</sup>	83.4 <sup>b</sup>
	High (20 %)	Control	69.9 <sup>a</sup>	67.2 <sup>a</sup>	69.9 <sup>a</sup>	72.9 <sup>a</sup>	119.7 <sup>a</sup>	53.7 <sup>a</sup>	38.8 <sup>a</sup>
		PL + SD	65.5 <sup>ab</sup>	63.1 <sup>a</sup>	62.9 <sup>ab</sup>	63.5 <sup>b</sup>	47.6 <sup>b</sup>	49.6 <sup>a</sup>	24.5 <sup>b</sup>
		PI + SD + GTE + GSE	61.0 <sup>b</sup>	60.9 <sup>a</sup>	62.1 <sup>b</sup>	55.9 <sup>c</sup>	46.3 <sup>b</sup>	23.1 <sup>a</sup>	20.9 <sup>b</sup>

T1: No chemical preservatives or plant extracts, T2: PL (2.0 %) + SD (0.15 %), T3: PL (0.15 %) + SD (0.11 %) + GTE (0.35 %) + GSE (0.22 %).

LSD to compare means in texture for different times at same meat and fat and PL- SD- GTE-GSE combinations = 22.69

LSD to compare means in  $a_w$  for same or different times at different meat or fat or PL- SD- GTE-GSE combinations = 22.65

**Table 3** Headspace hexanal (ppm) detected from GC-MS analyses in low and high fat chicken and turkey at 6<sup>th</sup> and 7<sup>th</sup> week of storage (4 °C)

Meat	Fat level	Treatment	6 <sup>th</sup>	7 <sup>th</sup>
Chicken	Low (3 %)	Control (T1)	13.59 <sup>b</sup>	26.6 <sup>b</sup>
		PL + SD (T2)	25.38 <sup>a</sup>	33.4 <sup>a</sup>
		PI + SD + GTE + GSE (T3)	ND	12.91 <sup>c</sup>
	High (20 %)	Control (T1)	50.27 <sup>b</sup>	65.61 <sup>b</sup>
		PL + SD (T2)	79.61 <sup>a</sup>	97.21 <sup>a</sup>
		PI + SD + GTE + GSE (T3)	ND	25.92 <sup>c</sup>
Turkey	Low (3 %)	Control (T1)	15.56 <sup>b</sup>	69.61 <sup>b</sup>
		PL + SD (T2)	33.90 <sup>a</sup>	75.95 <sup>a</sup>
		PI + SD + GTE + GSE (T3)	ND	14.34 <sup>c</sup>
	High (20 %)	Control (T1)	69.9 <sup>b</sup>	41.48 <sup>b</sup>
		PL + SD (T2)	86.24 <sup>c</sup>	95.48 <sup>d</sup>
		PI + SD + GTE + GSE (T3)	ND	29.11 <sup>bc</sup>

T1: No chemical preservatives or plant extracts, T2: PL (2.0 %) + SD (0.15 %), T3: PL (0.15 %) + SD (0.11 %) + GTE (0.35 %) + GSE (0.22 %).

ND – Not detected

LSD to compare means in hexanal content for different times same meat and fat and PL- SD- GTE-GSE combinations = 17.32

LSD to compare means in hexanal content for same or different times at different meat or fat or PL- SD- GTE-GSE combinations = 19.81

**Table 4a** Consumer sensory attributes of low fat turkey hotdogs recorded on 9-point hedonic scale

Sensory attribute <sup>2</sup>	Control	Test
Overall Impression <sup>2</sup>	5.3 <sup>a</sup>	5.7 <sup>a</sup>
Appearance <sup>2</sup>	4.9 <sup>a</sup>	5.0 <sup>a</sup>
Color <sup>2</sup>	4.7 <sup>a</sup>	4.4 <sup>a</sup>
Flavor <sup>2</sup>	5.6 <sup>a</sup>	5.9 <sup>a</sup>
Texture <sup>2</sup>	4.8 <sup>b</sup>	6.1 <sup>a</sup>
Juiciness <sup>2</sup>	5.7 <sup>a</sup>	6.1 <sup>a</sup>

<sup>1</sup>n = 56 panelists

Control = Potassium lactate (2.0 %) and sodium diacetate (0.15 %) used at commercial hotdog formulation levels.

Test = Hotdogs formulated with partial replacement of PL (1.5 %) and SD (0.11 %) with green tea (0.35 %) and grape seed extracts (0.22 %).

<sup>2</sup>9-point hedonic scale; 1 = Dislike extremely, 5 = Neither like or dislike, 9 = Like extremely

<sup>a-b</sup> Means within a row lacking a common superscript differ ( $P < 0.05$ ).

**Table 4b** Consumer sensory attributes of low fat turkey hotdogs recorded on 5-point JAR scale

Sensory attribute <sup>2</sup>	Control	Test
Color JAR <sup>3</sup>	2.0 <sup>a</sup>	2.0 <sup>a</sup>
Firmness JAR <sup>3</sup>	3.6 <sup>a</sup>	3.1 <sup>b</sup>
Overall Flavor JAR <sup>3</sup>	2.6 <sup>a</sup>	2.7 <sup>a</sup>
Juiciness JAR <sup>3</sup>	2.6 <sup>a</sup>	2.7 <sup>a</sup>
Purchase Intent JAR <sup>3</sup>	2.7 <sup>a</sup>	2.8 <sup>a</sup>

<sup>1</sup>n = 56 panelists

Control = Potassium lactate (2.0 %) and sodium diacetate (0.15 %) used at commercial hotdog formulation levels.

Test = Hotdogs formulated with partial replacement of PL (1.5 %) and SD (0.11 %) with green tea (0.35 %) and grape seed extracts (0.22 %).

<sup>2</sup>5-point Just About Right scale; 1=Too little, 3=Just about right, 5=Too much

<sup>a-b</sup> Means within a row lacking a common superscript differ ( $P < 0.05$ ).

**Table 5** Frequency (%) of consumer responses<sup>1</sup> for the attributes (overall impression, appearance, color, flavor, texture, and juiciness) of the low fat turkey hotdogs in the consumer sensory test

9-point hedonic scale	Overall Impression		Appearance		Color		Flavor		Texture		Juiciness	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Dislike Extremely (1)	3.85	1.92	3.85	3.85	9.62	5.77	1.92	0.00	7.69	0.00	0.00	0.00
Dislike very much (2)	9.62	5.77	9.62	7.69	3.85	9.62	3.85	3.85	11.54	5.77	0.00	1.92
Dislike moderately (3)	9.62	11.54	21.15	11.54	17.31	13.46	15.38	13.46	9.62	5.77	9.62	3.85
Dislike slightly (4)	13.46	9.62	11.54	23.08	23.08	36.54	11.54	11.54	25.00	9.62	17.31	13.46
Neither like nor dislike (5)	11.54	9.62	7.69	11.54	9.62	7.69	5.77	7.69	3.85	9.62	15.38	19.23
Like slightly (6)	13.46	13.46	21.15	11.54	11.54	5.77	23.08	15.38	11.54	23.08	23.08	15.38
Like moderately (7)	25.00	30.77	15.38	19.23	17.31	15.38	19.23	19.23	11.54	19.23	23.08	21.15
Like very much (8)	13.46	17.31	5.77	11.54	3.85	3.85	17.31	28.85	19.23	25.00	11.54	23.08
Like extremely (9)	0.00	0.00	3.85	0.00	3.85	1.92	1.92	0.00	0.00	1.92	0.00	1.92

<sup>1</sup>n = 56 panelists

Control = Potassium lactate (2.0 %) and sodium diacetate (0.15 %) used at commercial hotdog formulation levels

Test = Hotdogs formulated with partial replacement of PL (1.5 %) and SD (0.11 %) with green tea (0.35 %) and grape seed extracts (0.22 %).

**Table 6** Frequency (%) of consumer responses<sup>1</sup> for the Just-About-Right scale attributes (color, firmness, overall flavor, juiciness, purchase intent) of the low fat turkey hotdogs

<b>Attribute scale</b>	<b>Control</b>	<b>Test</b>
<b>Color</b>		
Much too light (1)	25.00	21.15
Too light (2)	55.80	59.62
Just about right (3)	17.30	15.38
Too dark (4)	1.90	3.85
Much too dark (5)	0.00	0.00
<b>Firmness</b>		
Much too firm (1)	0.00	0.00
Too firm (2)	3.85	9.62
Just about right (3)	42.31	76.92
Too soft (4)	40.38	9.62
Much too soft (5)	13.46	25.89
<b>Overall flavor</b>		
Much too strong (1)	9.52	5.77
Too strong (2)	34.62	30.77
Just about right (3)	48.08	55.77
Too weak (4)	9.62	1.92
Much too weak (5)	0.00	5.77
<b>Juiciness</b>		
Much too juicy (1)	3.85	1.92
Too juicy (2)	40.38	30.77
Just about right (3)	50.00	63.46
Too dry (4)	5.77	3.85
Much too dry (5)	0.00	0.00
<b>Purchase Intent</b>		
Definitely would purchase(1)	13.46	13.46
Probably would purchase (2)	30.77	25.00
May or may not purchase (3)	26.92	28.85
Probably would not purchase (4)	28.85	28.85
Definitely would not purchase (5)	0.00	3.85

<sup>1</sup>n = 56 panelists

Control = Potassium lactate (2.0 %) and sodium diacetate (0.15 %) used at commercial hotdog formulation levels

Test = Hotdogs formulated with partial replacement of PL (1.5 %) and SD (0.11 %) with green tea (0.35 %) and grape seed extracts (0.22 %).

APPENDIX I

SENSORY BALLOT USED FOR THE CONSUMER SENSORY ANALYSIS



UNIVERSITY OF ARKANSAS
DIVISION OF AGRICULTURE

To start the test, click on the Continue button below:

Product: Ready-To-Eat Turkey Hotdog

Panelist Code: \_\_\_\_\_

Panelist Name: \_\_\_\_\_

Question # 1 - Sample \_\_\_\_\_

Please observe and taste this sample. All things considered, which statement best describes your OVERALL IMPRESSION of this product?

Overall Impression

Dislike Extremely, Dislike Very Much, Dislike Moderately, Dislike Slightly, Neither Like Nor Dislike, Like Slightly, Like Moderately, Like Very Much, Like Extremely

Grid of 9 empty boxes for rating

Question # 2 - Sample \_\_\_\_\_

Which statement best describes the APPEARANCE of this product?

Appearance

Dislike Extremely, Dislike Very Much, Dislike Moderately, Dislike Slightly, Neither Like Nor Dislike, Like Slightly, Like Moderately, Like Very Much, Like Extremely

--	--	--	--	--	--	--	--	--

**Question # 3 - Sample \_\_\_\_\_**

Which statement best describes your impression of the **COLOR** of this product?

**Color**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
----------------------	----------------------	-----------------------	---------------------	--------------------------------	------------------	--------------------	----------------------	-------------------

--	--	--	--	--	--	--	--	--

**Question # 4 - Sample \_\_\_\_\_**

**Color Intensity**

Considering only the **COLOR**, which statement below describes your impression of this product?

Much too Pale	Too Pale	Just About Right	Too dark	Much too dark
---------------	----------	------------------	----------	---------------

--	--	--	--	--

**Question # 5 - Sample \_\_\_\_\_**

Which statement best describes your impression of the **FLAVOR** of this product?

**Flavor**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
----------------------	----------------------	-----------------------	---------------------	--------------------------------	------------------	--------------------	----------------------	-------------------

--	--	--	--	--	--	--	--	--

**Question # 6 - Sample \_\_\_\_\_**

**Flavor Intensity**

Considering only the **FLAVOR**, which statement below describes your impression of this product?

Much too weak	Too weak	Just About Right	Too strong	Much too strong
---------------	----------	------------------	------------	-----------------

--	--	--	--	--

**Question # 7 - Sample \_\_\_\_\_**

Which statement best describes your impression of the **TEXTURE (MOUTHFEEL)** of this product?

**Texture (Mouth feel)**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

**Question # 8 - Sample \_\_\_\_\_**

**Texture Intensity**

Considering only the **TEXTURE**, which statement below describes your impression of this product?

Much too soft	Too soft	Just About Right	Too firm	Much too firm
<input type="text"/>				

**Question # 9 - Sample \_\_\_\_\_**

Which statement best describes your impression of the **JUICINESS** of this product?

**Juiciness**

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like Nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

**Question # 10 - Sample \_\_\_\_\_**

Considering only the **JUICINESS**, which statement below describes your impression of this product?

**Juiciness Intensity**

Much too dry	Too dry	Just About Right	Too juicy	Much too juicy
<input type="text"/>				

**Question 11 Sample \_\_\_\_\_**

Do you perceive/feel an **AFTERTASTE** ?

Yes or No

If yes, Please describe.....

----- End of Taste Test -----

**Question # 12**

All things considered, which sample did you prefer **OVERALL** ?

**Question # 13**

In Sample XXX, we added natural plant extracts to reduce the concentration of chemical preservatives to extend the shelf life [Safe for human consumption (FDA Approved, Generally Recognized As Safe; GRAS)].

Knowing this.....

How likely would you be to purchase this sample, if it was available at a reasonable price in a store you normally shop?

**Purchase Intent**

Definitely Would  
Not Buy

Probably Would  
Not Buy

May or May Not  
Buy

Probably Would  
Buy

Definitely Would  
Buy