

**CONTROL OF *LISTERIA MONOCYTOGENES* ON READY-TO-EAT FOODS  
USING EDIBLE ANTIMICROBIAL COATINGS AND FILMS**

by

Zheng Jiang

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Zheng Jiang

Approved: \_\_\_\_\_  
Haiqiang Chen, Ph.D.  
Professor in charge of thesis on behalf of the Advisory Committee

Approved: \_\_\_\_\_  
Jack Gelb, Ph.D.  
Chair of the Department of Animal and Food Sciences

Approved: \_\_\_\_\_  
Robin Morgan, Ph.D.  
Dean of the College of Agriculture and Natural Resources

Approved: \_\_\_\_\_  
Debra Hess Norris, M.S.  
Vice Provost for Graduate and Professional Education

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## TABLE OF CONTENTS

LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
ABSTRACT .....	viii
Chapter	
1 INTRODUCTION .....	1
2 LITERATURE REVIEW .....	6
<i>Listeria monocytogenes</i> .....	6
History .....	6
Genus and serotyping .....	6
Morphology .....	7
Growth Requirements.....	7
Food mediated hurdles to <i>Listeria monocytogenes</i> growth.....	8
Human Listeriosis.....	10
Prevalence of <i>Listeria monocytogenes</i> in fishery products and meat products .....	12
Active packaging.....	15
Antimicrobial packaging .....	15
Film.....	17
Carriers .....	18
Antimicrobials .....	20
References .....	24
3 CONTROL OF <i>LISTERIA MONOCYTOGENES</i> ON COLD-SMOKED SALMON AND HAM STEAKS USING CHITOSAN BASED ANTIMICROBIAL COATINGS AND FILMS .....	37
Abstract.....	37
Introduction .....	38
Materials and Methods .....	40
Results and Discussion.....	43

	References .....	51
	Figures .....	55
	Tables .....	57
4	CONTROL OF <i>LISTERIA MONOCYTOGENES</i> ON ROASTED TURKEY USING ANTIMICROBIAL EDIBLE COATINGS DURING FREEZING AND/OR REFRIGERATED STORAGE .....	60
	Abstract .....	60
	Introduction .....	61
	Materials and Methods .....	63
	Results and Discussion .....	67
	References .....	74
	Figures .....	78
	Tables .....	82
5	FUTURE RESEARCH .....	89
	References .....	91

## LIST OF TABLES

Table 3.1 Antimicrobial concentrations (% weight of antimicrobials/weight of smoked salmon) in chitosan coatings and films applied onto cold-smoked salmon.....	57
Table 3.2 Antimicrobial concentrations (% weight of antimicrobials/weight of smoked salmon) in chitosan coatings and films applied onto ham steaks .....	58
Table 3.3 Effect of chitosan-based edible films and coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged cold-smoked salmon stored at 4°C for 30 days .....	59
Table 4.1 Antimicrobial concentrations (%weight of antimicrobials/weight of roasted turkey) in varied coatings applied onto roasted turkey.....	82
Table 4.2 Antimicrobial concentrations (%weight of antimicrobials/weight of roasted turkey) in pectin coatings applied onto roasted turkey.....	83
Table 4.3 Effect of pectin-based edible coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged roasted turkey stored at up to 4 week frozen temperature followed up to 8 week refrigerated temperature storage. ....	84
Table 4.4 Effect of pectin-based edible coatings incorporating GRAS antimicrobials on the growth of aerobic and anaerobic microflora on vacuum packaged roasted turkey stored at up to 4 week frozen temperature followed up to 8 week refrigerated temperature storage .....	87

## LIST OF FIGURES

Figure 3.1	Effect of chitosan-based edible coatings incorporating GRAS antimicrobials at low levels on the growth of <i>Listeria monocytogenes</i> on vacuum packaged ham steaks stored at 4°C.....	55
Figure 3.2	Effect of chitosan-based edible coatings incorporating GRAS antimicrobials at high levels on the growth of <i>Listeria monocytogenes</i> on vacuum packaged ham steaks stored at 4°C.....	56
Figure 4.1a	Effect of starch-based edible coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged roasted turkey stored at 4°C .....	78
Figure 4.1b	Effect of chitosan-based edible coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged roasted turkey stored at 4°C .....	79
Figure 4.1c	Effect of alginate-based edible coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged roasted turkey stored at 4°C .....	80
Figure 4.1d	Effect of pectin-based edible coatings incorporating GRAS antimicrobials on the growth of <i>Listeria monocytogenes</i> on vacuum packaged roasted turkey stored at 4°C .....	81

## ABSTRACT

Growth of *Listeria monocytogenes* in processed fishery products and meat products such as cold-smoked salmon, ham steaks and roasted turkey has become a major concern and an important food safety issue. The food industry therefore has incorporated hurdles to inactivate *Listeria monocytogenes*. The objective of this research was to examine if antimicrobial packaging could be used as a hurdle to inhibit *Listeria monocytogenes* on the surface of these food. Cold-smoked salmon samples were surface-inoculated with a five-strain cocktail of *Listeria monocytogenes* to a final concentration of  $4.4 \log_{10}$  CFU/cm<sup>2</sup> and coated with chitosan coating solution or wrapped in chitosan films with or without the antimicrobials sodium lactate (SL), sodium diacetate (SD), potassium sorbate (PS)). The samples were vacuum packaged and stored at 4° C for 30 days. The chitosan coatings, with or without the antimicrobials, were consistently more effective against *Listeria monocytogenes* than chitosan films of the same composition. Chitosan films containing 1.2% SL/0.25% SD or 2.4% SL and chitosan coatings containing 1.2% SL/0.25% SD or 0.15% PS/0.125% SD displayed the greatest anti-listerial activity. This study showed that chitosan-based edible coatings and films hold promise and can potentially assist fishery industries in their efforts to control *Listeria monocytogenes*.

We then subsequently tested the effectiveness of chitosan coatings incorporating selected binary antimicrobial combinations (PS/SD, PS/SL, SL/SD, SD/SL) on ham steaks and showed that 1.2% SL/0.25%SD resulted in *Listeria monocytogenes* populations that were  $\sim 2.3 \log_{10}$  CFU/cm<sup>2</sup> lower relative to the

control. The study shows that chitosan-based edible coatings hold promise and can potentially assist in their efforts to control *Listeria monocytogenes*.

The objective of the following study was to evaluate the efficacy of four polysaccharide material based edible coatings (starch, chitosan, alginate and pectin) incorporating six Generally Recognized as Safe (GRAS) antimicrobials, sodium lactate (SL), sodium diacetate (SD), OptiForm<sup>®</sup> PD4, NovaGARD<sup>™</sup>CB1, Protect M and Guardian<sup>™</sup> NR 100 against *Listeria monocytogenes* on roasted turkey. Roasted turkey were surface-inoculated with a five-strain cocktail of *Listeria monocytogenes* to a final concentration of  $\sim 3 \log_{10}$  CFU/cm<sup>2</sup> and then coated with different coating solutions with or without antimicrobials. The samples were vacuum packaged and stored at 4° C for up to 8 weeks. Pectin coatings with antimicrobials were most efficient against *Listeria monocytogenes*. Therefore, these types of coatings were employed in the next phase study, which investigated the effect of frozen storage. Pectin coating containing antimicrobials significantly lowered the *Listeria monocytogenes* population after four weeks of frozen and eight weeks of refrigerated storage. OptiForm (2.5%) displayed the greatest antilisterial activity and achieved a 1.2- $\log_{10}$  CFU/cm<sup>2</sup> reduction. Although the time of frozen storage did not have a statistically significant influence on *Listeria monocytogenes* populations, *Listeria monocytogenes* counts in samples stored frozen for four weeks were lowest by the end of storage. Growth of aerobic and anaerobic microbes was slowed down using pectin coatings containing antimicrobials after 4-week frozen and subsequent 8-week refrigerated storage. This study showed that pectin-based edible coatings hold promise in controlling *Listeria monocytogenes* in the meat industry, and freezing can potentially enhance their effectiveness.

## Chapter 1

### INTRODUCTION

#### *Listeria monocytogenes*

*Listeria monocytogenes* is a gram-positive, non-spore-forming pathogenic bacterium that can cause infection in humans after consumption of contaminated food (Jay et al., 2005). It has been recognized as a serious human pathogen since 1929 and as a food-borne pathogen since 1981. *Listeria monocytogenes* exists ubiquitously in nature and can grow widely in processing environments, which causes it to be very difficult to eliminate from a food production facility. The organism's optimum growth temperature is 30-37°C; however, it is able to grow at temperatures as low as 1°C and as high as 45°C (Hitchins, 1996). It can grow during refrigeration where other competing organisms may not (Swaminathan et al., 2007). The symptoms of the disease can range from mild non-invasive listerial gastroenteritis to severe and sometimes even life-threatening invasive listeriosis (CFSAN, 2003; CFSAN, 2008). Fetuses and neonates are at high risk of infection if the mother is exposed to *Listeria monocytogenes* during pregnancy. The disease may even cause spontaneous abortions or stillbirths (Anonymous, 2003). The elderly and immune compromised patients with hematologic malignancies, with AIDS, receiving organ transplant, or receiving corticosteroid therapy also have great risk of experiencing listeriosis due to their weakened immune systems. The fatality rate is high for invasive listeriosis, around 20% to 30% (USFDA/FSIS, 2003; CFSAN, 2008). The Center for Disease Control and Prevention estimated that 500 deaths and approximately 2,500 serious illnesses

occur due to listeriosis each year in the United States (CFSAN, 2003) and that the fatality rate in immune-compromised individuals was 28% (CDC, 2005). A number of these cases are part of outbreaks affecting a large number of consumers. Foods associated with these outbreaks are regarded as *Listeria monocytogenes* growth supporters and most of these are ready-to-eat (RTE) foods such as deli meats, frankfurters, butter, fresh soft cheese and coleslaw (Levine, 2001; CFSAN, 2008). *Listeria monocytogenes* is widespread in the environment (NACMC, 1991; Fenlon et al., 1996; Fenlon, 1999), and can grow well in the soil, water, sewage and decaying vegetation. It can be easily found in humans, domestic animals and processing environments. Potential sources of *Listeria monocytogenes* are ingredients, processing aids, contact surfaces for RTE foods, but also surfaces that do not contact foods, and processing plant environments (Tompkin et al., 1999; USFDA/FSIS, 2003).

The U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) were prompted by several large listeriosis outbreaks in the 1980's to establish a "zero tolerance" policy and FSIS (2003) published a rule that required establishments to develop effective ways to control *Listeria monocytogenes* in RTE food such as deli meats and frankfurters due to a very high risk associated with consumption of deli meats and frankfurters (not reheated) annually and a high risk associated with these products per serving (Anonymous, 2003; Swaminathan et al., 2007). However, it is challenging to completely eliminate *Listeria monocytogenes* from processed RTE foods. A survey by the National Food Processors Association (NFPA) (Gombas et al., 2003) showed that the prevalence of *Listeria monocytogenes* in RTE foods was 1.8%, with levels in contaminated samples ranging from as low as <0.3 MPN/g to as high as  $1.5 \times 10^5$

colony forming units (CFU)/g.

The incidence of *Listeria monocytogenes* in cold-smoked salmon and cooked fish products has been reported to range from 6 to 36% and a draft risk assessment by FDA and USDA estimated that 15% of all smoked fish is contaminated with *Listeria monocytogenes* (Anonymous, 2001; Embarek 1994; Gombas et al., 2003), which has raised concern about the survival and growth potential of the organism in seafoods before consumption. However, the rapid cooling reduces potential growth of the pathogenic organisms in meat products. Inhibition of growth of *Listeria monocytogenes* in RTE food cannot be guaranteed using current combinations of NaCl and low temperatures; however, growth can be prevented by freezing, by addition of certain additives, by use of bioprotective bacterial cultures or by heating to a high enough temperatures (CFSAN, 2008). There may be post-processing contamination of the food product after the treatment and subsequent growth (Zhu et al., 2005). Due to the risk of post-processing contamination, post-lethality treatments are needed to inhibit growth of *Listeria monocytogenes* in RTE foods.

Although *Listeria monocytogenes* is a hardy organism, it can be inhibited by a number of measures. *Listeria monocytogenes* does not grow when the pH value is less than or equal to 4.4, the water activity is less than or equal to 0.92 or the food is frozen (Sorrells et al., 1989; Tienungoon et al., 2000; IFT/FDA, 2001). Low temperature decreases its growth rate (Lou and Yousef, 1999; IFT/FDA, 2001; USFDA/FSIS, 2003) and some antimicrobial substances have synergistic inhibitory effects with other parameters, such as the pH, water activity, the presence of preservatives and processing temperature (USFDA/FSIS, 2003).

Generally, a control measure is considered effective if less than one log

increase in the number of *Listeria monocytogenes* is realized in replicate growth study trials with the food (Scott et al., 2005). Complete elimination of *Listeria monocytogenes* from RTE foods would be desirable, but achieving this goal is challenging (Yang et al., 2005). Because the pathogen is ubiquitous in the environment and able to withstand various environmental and processing stresses (Wang et al., 1994); characteristics that render its presence in food processing environments undesirable as well as inevitable (Tompkin, 2002). Hygienic and sanitation practices applied in processing plants are often insufficient to prevent contamination of processed products (Farber et al., 1999). The pathogen contaminates products mainly after thermal processing. And contaminating *Listeria monocytogenes* can be resistant to many food preservation methods and increase to high numbers during refrigerated storage and under low oxygen tension (Farber et al., 1999; Glass et al., 1989; Grau et al., 1992; Samelis et al., 1999).

#### Edible films and coatings

The demand by consumers for enhanced food safety and improved food product freshness, the need by food processors for new storage techniques and the concerns over disposal of non-renewable packaging materials have increased interests in the development and implementation of new packaging techniques (Gennadios et al., 1997). Food packaging functions mainly in two ways, to maintain optimal internal gaseous atmosphere and to resist external deteriorative influences (Srinivasa and Tharanathan, 2007). Active food-packaging concepts include several additional functions compared with traditional passive packaging materials. As an artificial barrier to gaseous diffusion, active packaging achieved by coatings and films provide the means of achieving quality improvement and physiological and pathological

disorders reduction (Smith et al., 1987). The term coating is defined to be a thin layer of a foreign material applied to the surface of the food, as an additional covering over the natural protective cover. The coating may be prepared by dipping, drenching, spraying or by hand with a tool. However, the nonuniformity of coating may induce progression of anaerobiosis and spoilage of fruits or vegetables (Dhalla, 1998). Films are extruded materials that are used to surround the produce as wraps, they are capable to restrict water loss during the long time storage (Marcellin, 1974).

The coatings can become part of an antimicrobial intervention (“hurdle”). Cagri (2004) defined edible coatings as continuous matrices that could be prepared from proteins, polysaccharides and lipids. Polysaccharides function as one of the most popular coating materials. Generally, high moisture gelatinous polysaccharide coatings such as starch and its derivatives, alginates, chitosan and pectinates retard moisture loss from food due to their hydrophilic nature and therefore act more as sacrificing agents than moisture barriers (Kester and Fennema, 1986).

The objectives of this research were to (i) determine the antilisterial efficacy of chitosan-based films and coatings incorporating antimicrobials on cold-smoked salmon and ham steaks during refrigerated storage (ii) determine the antilisterial efficacy of varied coatings incorporating GRAS antimicrobials on cooked turkey during refrigerated and freezing storage (iii) investigate the possible synergistic antimicrobial effects of edible coatings incorporating antimicrobial treatments in combination with frozen storage to inhibit growth of *Listeria monocytogenes* on cooked turkey.

## Chapter 2

### LITERATURE REVIEW

#### *Listeria monocytogenes*

##### History

In 1924, the bacterium *Listeria monocytogenes* and the disease listeriosis were recognized in laboratory animals in Cambridge for the first time (Murray et al., 1926). Later, people found that the disease also affected human beings. In the 1980's a rise in the number of listeriosis in several countries was observed and the food-borne transmission and the distribution of the bacterium has now been firmly established using improved detection methods (McLauchlin, 1996a, b). The morbidity, mortality, epidemiology, pathogenicity and mechanisms of listeriosis are now well established, but research on how to reduce or eliminate this bacterium from foods is still required.

##### Genus and serotyping

The genus *Listeria* includes six species, and these comprise *Listeria monocytogenes*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri*, *Listeria ivanovii* and *Listeria grayi* (McLauchlin and Jones, 1999). *Listeria monocytogenes* is the major pathogenic species in both animals and man (McLauchlin and Jones, 1999).

Antigenic types are distinguished by somatic (O) and flagellar (H) antigens. Based on these antigens, strains of *Listeria monocytogenes* are subdivided

into serotypes: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4% 4ab, 4b, 4c, 4d, 4e, 7 (McLaughlin, 1987), 4f, 4g, 5 and 6 (Ralovich, 1984). McLaughlin (1987) found out that for the 722 human cases of listeriosis in the US, 59% were due to serotype 4b and 18% were caused by serotype 1/2a.

### **Morphology**

*Listeria monocytogenes* is a small, facultatively anaerobic, gram-positive, catalase-positive, oxidase-negative, acapsular, nonsporulating bacterium. It grows readily on blood agar, producing a narrow zone of incomplete  $\beta$ -hemolysis caused by a soluble hemotoxin (Seeliger, 1961; Farber and Peterkin, 1991). The diphtheroid-like rod measures 1.0 to 2.0  $\mu\text{m}$  by 0.5 $\mu\text{m}$ . *Listeria* is motile, predominantly by polar peritrichous flagella, and exhibit characteristic “tumbling motility” at 20 to 25°C (Grey, 1966). Colonies appear blue or green whereas others appear orange or yellow when grown on blood-free agar and viewed by the Henry method of oblique lighting at a 45-degree angle. When cultured on nutrient agar (after 24 hours of incubation) colonies are round, 0.5-1.5 mm in diameter, translucent and with a smooth glistening surface (S-form). During prolonged incubation for 3-7 days, colonies may appear rough (R-form) and 3-5 mm in size.

### **Growth Requirements**

*Listeria* is not fastidious. They can survive in feces, milk, soil, water, silage, and on plants. Growth occurs between 0.5 (Juntilla et al., 1988) and 45°C (Petron and Zottola, 1989). *Listeria monocytogenes* grows optimally at 30-37°C. It can be enriched in mixed cultures by incubation in the cold since it is able to grow faster than most other organism at low temperature (Lorber, 2007). Bajard et al. (1996) revealed that *Listeria monocytogenes* can grow at temperatures as low as -2°C in

laboratory media. This was in line with Bell's research (1995) in vacuum-packaged smoked blue cod, where growth was observed at -1.5°C.

*Listeria* can multiply in as high as 10% sodium chloride (Seelinger, 1961). It grows best at neutral to slightly alkaline conditions but multiplies readily from pH 4.6 to as high as 9.6 with an optimum at 7.1 (Seelinger, 1961; Gray and Killinger, 1966; AFSSA, 2000). *Listeria monocytogenes* does not grow when the pH value is less than or equal to 4.4, the water activity is less than or equal to 0.92 or the food is frozen (Sorrells et al., 1989; Tienungoon et al., 2000; IFT/FDA, 2001).

*Listeria monocytogenes* requires biotin, riboflavin, thiamine, thioctic acid, and some amino acids for growth (Pearson and Marth, 1990). The energy sources are mainly from carbohydrate and/or glucose by glycolysis, with the mainly end product lactic acid (Jones, 1974; Pine et al., 1989; Premaratne et al., 1991). The organism can grow in the presence of CO<sub>2</sub> at low temperatures. Growth is enhanced with reduced oxygen and 5 to 10% CO<sub>2</sub>, but CO<sub>2</sub> concentrations above 70% can inhibit the growth of *Listeria monocytogenes* at temperatures lower than 7°C (Wimpfheimer et al., 1990).

### **Food mediated hurdles to *Listeria monocytogenes* growth**

#### Temperature

*Listeria monocytogenes* grows in refrigerated foods (Augustin 1999), and for most time, the temperature of home refrigerators is often closer to 9° C (Sergelidis et al. 1997), which favors its growth. Even when initial contamination is low, the organism in food involved in listeriosis outbreaks can always multiply during refrigeration and reach levels significantly > 100 CFU/g (AFSSA 2000).

*Listeria monocytogenes* has higher thermotolerance than other non-spore-forming food pathogens (Fleming et al., 1985). And exposure to a variety of

environmental stresses like heating at sublethal temperatures or osmotic and acidic shocks may increase its thermotolerance (Fleming et al., 1985). The heat resistance of *Listeria monocytogenes* depends on both growth conditions and environmental factors during processing. Some important environmental factors influencing the heat resistance of *Listeria monocytogenes* include temperature, pH, acid type, and fat level. Linton et al. (1992) observed that higher sublethal temperatures (50-60° C) and longer treatments increased its resistance more.

#### pH

The usual pH of meats (5.0–5.5) can induce an acid tolerance response in *Listeria monocytogenes* (Faleiro et al. 2003), and this acid adaptation can induce cross-protection against several other damaging factors including osmotic stress, although the cross-stress protection varies depending on the strain (O’Driscoll et al., 1996; Vasseur et al., 2001; Faleiro et al., 2003). Other sublethal stresses such as osmotic, heat and low temperatures may not lower the acid sensitivity of *Listeria monocytogenes* (Koutsoumanis et al., 2003).

#### NaCl

*Listeria monocytogenes* may also encounter high salt concentrations in dried sausages, raw dried ham or cooked meats (Foegeding et al. 1992; Dabin and Jussiaux 1994). Strains of *Listeria monocytogenes* with acid adaptation may become cross-protected against osmotic stress (O’Driscoll et al. 1996; Vasseur et al. 1999). However, osmotic adaptation does not affect sensitivity of other damaging factors (Lou and Yousef 1997).

#### Water activity

Water activity is strongly associated with the pH and salt content. During

the drying of sausage products, both the water holding capacity and pH decreased (Tyopponen et al. 2003). The addition of salt to the food limits water activity, thus controlling the growth of *Listeria monocytogenes* (Lücke, 1985). Insufficient drying of products resulting in a water activity of above 0.92 is associated with a high risk of *Listeria monocytogenes*.

#### Lactic acid bacteria (LAB)

LAB produce lactic acid, resulting in low pH values (Leistner 1995), which can effectively eliminate or inhibit spoilage and pathogen contaminants (Tyopponen et al. 2003). Meat manufacturers regard LAB useful organisms and may add LAB starters and glucose (the energy source for LAB) to meat matrix to control the drop in pH (Ross et al. 2002). Many bacteria in the LAB family produce bacteriocins, which generally depolarize the target cell membrane or inhibit cell wall synthesis (Ross et al. 2002). Some of the bacteriocins affect *Listeria*.

Beside useful LAB, there is a group of bad natural microflora composed of spoilage bacteria including hetero- fermentative LAB, pseudomonads and enterobacteria (Samelis et al. 1998), whose presence in raw meats lead to spoilage (Dabin and Jussiaux 1994) and a raise in the number of background microflora.

### **Human Listeriosis**

*Listeria monocytogenes* causes generally prevalent fever and flu-like symptoms including, nausea, vomiting and diarrhea (CFSAN, 2000) to most people or some minor skin infections particularly to farmers and veterinarians handling bovine calvings or abortions (McLauchlin and Low, 1994); although relatively rare, it may also cause meningitis or encephalitis. Listeriosis results in spontaneous abortion, still birth or even infection in newborn in pregnant women (Seeliger and Finger, 1983).

The infectious dose is unknown and varies due to different level of host susceptibility. Most cases happened to people with compromised immune systems such as patients with cancer, AIDS, diabetes, kidney-disease, the elderly (>65) or pregnant women (CDC, 2000). Besides, virulence of the microorganism; type and amount of food consumed; and concentration of the pathogen in food are also important factors (Risk Assessment Drafting Group, 2004).

During 1980s, a link between listeriosis and consumption of contaminated foodstuffs was established for a number of listeriosis outbreaks. In 1981, the first clearly established community outbreak occurred in the Maritime Provinces in Canada (Schlech et al., 1983). Seven adults and 34 pregnancies became ill due to contaminated coleslaw. Contaminated pasteurized milk caused an outbreak in Massachusetts in 1983 (Fleming et al., 1985) and Mexican-style cheese was associated with further listeriosis outbreaks in California in 1985, resulting in 29 deaths and more than 86 illnesses (CDC, 1985). The CDC (2000) reported that in 1988, 40 illnesses were linked to environmentally contaminated deli meats and frankfurters; in 2000, 29 illnesses were associated with deli turkey meat contaminated with this organism. The Centers for Disease Control (CDC, 2000) estimated 2,500 cases with 500 deaths per year in the United States.

Other countries met with the same serious problems. In Switzerland, outbreaks due to contaminated soft cheeses occurred between 1983-1987 (Piffaretti et al., 1989), and contaminated paté caused infections from 1987 to 1989 in the United Kingdom (McLauchlin et al., 1991; Gilbert et al., 1993). A similar outbreak occurred in Western Australia in 1990 and France experienced an outbreak due to contaminated jellied pork tongues and pork 'rillettes' in 1992. Although awareness of contamination

and infection and the development of new techniques enhanced diagnosis rates, the incidence of listeriosis still increased in many western countries. In addition, human listeriosis outbreaks have been linked to consumption of contaminated Hispanic-style cheese in 1985 and 2000 in the United States (Linnan et al., 1988; MacDonald et al., 2005), contaminated butter in Finland (Lyytikäinen et al., 2000) and recent cases associated with fresh cheese (Carrique-Mas et al., 2003). It is interesting to point out that listeriosis outbreaks are rarely reported in Africa, Asia and South America (Low and Donachie, 1997).

Besides the predominant food-borne transmission, nosocomial infection and person-to-person spread are also means responsible for sporadic listeriosis cases. Moreover, veterinarians recognized close association between listeriosis and silage feeding in ruminants (Kendra et al., 2005).

However, the incidence of listeriosis has been declining in most industrialized countries during the past decade. The incidence of sporadic listeriosis declined by 40% between 1996-1998 and 2004 to 2.7 cases per million persons in the United States (CDC, 2004). This is most likely because of the aggressive implementation of *Listeria* control hurdles by the food industry with introduction of HACCP principles to guide food manufacturing, and the implementation and enforcement of microbiological criteria for *Listeria monocytogenes* in food with a zero or low tolerance in most countries for ready-to-eat foods that support growth of the organism, e.g. deli meat, dairy products or smoked fish.

### **Prevalence of *Listeria monocytogenes* in fishery products and meat products**

In 1983, epidemiological and laboratory investigations conclusively demonstrated transmission of *Listeria monocytogenes* by food contamination for the

first time (Schlech et al. 1983). A variety of different food items such as raw and processed meats, seafood, soft cheese, raw milk and fresh vegetables have been linked to both sporadic cases and outbreaks of listeriosis (AFSSA 2000; FICT 2002). *Listeria monocytogenes* is of particular concern in fishery products and meat products. The USA established “zero” tolerance criteria for food products, which means that no *Listeria monocytogenes* can be found in 25 g of a food product (Shank et al. 1996). Canada and Europe also had a zero tolerance level recommendations for *Listeria monocytogenes* according to the foodstuff.

In meat products, the incidence of *Listeria monocytogenes* is generally low (Encinas et al. 1999; AFSSA 2000; FICT 2002), and epidemiological data on foods involved in listeriosis outbreaks suggest that infections occurred when the organism had multiplied to levels significantly >1000 CFU/g (Ross et al. 2002; Risk Assessment Drafting Group 2004). The probability of infection with *Listeria* is thought to be very low if the contamination level was less than 100 CFU/g. In other words, it is possible that in some food products where *Listeria* is unable to grow, a level of no more than 100 CFU/g *Listeria monocytogenes* could be acceptable, but a zero tolerance would be necessary for foods that function as supporters for the growth of the bacteria and have extended shelf-lives (AFSSA 2000).

Meat and processed meat products are of particular concern with respect to *Listeria monocytogenes* contamination mainly because *Listeria monocytogenes* can multiply at refrigerated temperature and grow for long periods of time in food processing environments. The main paths of contamination are from the raw ingredients, the manufacturing process with insufficient sterilization, and contact with unprocessed contaminated raw materials, people and facilities (Samelis et al., 1998;

Chasseignaux et al., 2001).

Studies have documented the prevalence of *Listeria monocytogenes* on meat products. An incidence level of 6.7% in sliced vacuum packaged ready-to-eat meat and poultry products has been observed (Samelis and Metaxopoulos, 1999). In the US, 22.9% ground pork meat products and sausages sampled were contaminated with *Listeria monocytogenes* (Duffy et al., 2001). Three major *Listeria monocytogenes* outbreaks occurred in the RTE meat industry. In 1998–1999, an outbreak of listeriosis by hot dogs and deli meats resulted in 101 illnesses and 21 deaths in 22 states (FSIS, 1999). In 2000, RTE deli turkey meat contaminated by *Listeria monocytogenes* resulted in 29 illness and 4 deaths in 10 states (Hurd, et al., 2000). In 2002, the third outbreak associated with contaminated RTE deli turkey meat resulted in 63 illnesses, 8 deaths, and 3 miscarriages/stillbirths in 8 states (CDC, 2002). In each case, the contamination occurred during post-processing period (Office of the Federal Register, 2001).

Fishery products are also susceptible to *Listeria monocytogenes* contamination. Contaminated cold-smoked rainbow trout was caused five listeriosis cases in Finland, and gravid, cold-smoked or hot-smoked rainbow trout were suspected to be the source of a few cases in Sweden (Ericsson et al., 1997; Miettinen et al., 1999). A survey revealed that in six United States smoke houses, 50 to 100% of sampled products were contaminated with *Listeria monocytogenes* (Eklund and others, 1995). Heinitz and Johnson (1998) reported that 17.5% of cold-smoked fish (291 samples) and 8.1% of hot-smoked fish (234 samples) from the United States contained *Listeria monocytogenes*, and 7.3% of 96 cold-smoked fish samples from 5 United States smoke houses were positive (Norton et al., 2001). Smoked salmon are therefore

considered to be high-risk products for human listeriosis, and *Listeria monocytogenes* contamination is thus of a great concern for the smoked fish industry (Gombas et al., 2003).

### **Active packaging**

Active packaging technologies are being developed as a result of several driving forces, such as increased demand from consumers for minimally processed healthy and safe foods, globalized retail and distribution practices, automatic handling systems and new distribution trends with emphasis on Internet shopping (Vermeiren et al., 1999; Sonneveld, 2000). Active packaging is an innovative concept involving the interaction of package, product and environment. It could be defined as a type of packaging that changes the condition of the packaging to prolong shelf-life, and to enhance the safety and sensory properties of the product while maintaining the quality of the food (Labuza and Breene, 1989; Vermeiren et al., 1999; Suppakul et al., 2002). Foodborne microbial outbreaks call for a generation of food packaging including materials with antimicrobial properties. This antimicrobial food packaging technology could act to inhibit or retard the growth of microorganisms, reduce the risk from pathogens and it is a most promising version of active packaging system (Flores et al., 1997).

### **Antimicrobial packaging**

Antimicrobial food packaging materials impart antimicrobial effectiveness to extend the lag phase and reduce the growth rate of microorganisms in order to extend shelf life and to maintain product quality and safety (Han, 2000). They are supposed to have self-sterilizing abilities due to their antimicrobial effectiveness

(Hotchkiss, 1997). Food antimicrobial packaging can be obtained by incorporating or immobilizing antimicrobial agents into the packaging materials or by modifying or coating the surface of the food product, especially those smooth surfaces that come in contact with the inner surface of the package directly. Generally, antimicrobial packaging is classified in several types/forms: 1. Addition of sachets/pads containing volatile antimicrobial agents into packages; 2. Incorporation of volatile and non-volatile antimicrobial agents directly into polymers; 3. Coating or adsorbing antimicrobials onto polymer surfaces; 4. Immobilization of antimicrobials to polymers by ion or covalent linkages; 5. Use of polymers that are inherently antimicrobial (Appendini and Hotchkiss, 2002). It is hard to use a single antimicrobial agent to cover all the requirements for food preservatives, and it is important to develop antimicrobial systems that have as small an effect on the physical and mechanical properties of the food product. Therefore, the polarity and molecular weight of the additive have to be taken into consideration during incorporation of additives to make sure they are compatible with of the carrier. The ionic charge and solubility of different antimicrobials may affect the diffusion rates (Cooksey, 2000). Different foods with different biological and chemical characteristics stored under different temperatures may also affect the activity of antimicrobial packages. The protective action of antimicrobial packaging can deteriorate at high temperature due to high diffusion rates, that cannot be maintained throughout the shelf life of the product (Vojdani and Torres, 1989a, b; Wong et al., 1996; Cooksey, 2000).

Different countries have established different approval levels for organic acids, bacteriocins and volatile compounds. The FDA has approved some natural derived compounds as additives for certain foods (Brody et al., 2001) but declined the

use of Allyl isothiocyanate (AIT) which is potential to be contaminated while in Japan naturally derived AIT is approved into use. However, no specific regulation exists for active packaging. No regulations specifically for active packaging have been published in Europe so far. The overall migration limit of the additives into the food from the active package should not exceed 60 mg/kg due to European legal limits for migration from food packaging material, which is incompatible with the mechanism and purpose of the packaging system that expresses its effectiveness by releasing active ingredients into the food (Suppakul et al., 2002). Therefore, a new food packaging regulation is needed (Van Beest, 2001), to make the application of antimicrobial packaging a promising potential technology.

The enlarged world wide transportation and distribution mode, and the increasing demands from consumers for fresh and safe products presages a bright future for antimicrobial packaging (Floros et al., 1997). Currently, commercialized packages are limited, most of which are applied onto high-value food (Cooksey, 2000) such as chilled and frozen meats, tree nuts, and poultry meat.. There appears a need for scientists and technologists to pay more attention to the preservation efficacy of this type of packaging, to identify the types of foods that could benefit most and to control and/or decrease the cost (Han, 2000). Moreover, the odor/flavor should be taken into consideration when transferring additives into food products.

### **Films**

The use of the antimicrobial film is suitable especially for solid food products where superficial contaminants come immediately in contact with the antimicrobial film. A number of plastic and biodegradable film materials containing antimicrobials aiming to protect food against surface contamination/spoilage have been

developed and the mechanical and barrier properties of some biopolymer films have been analyzed (Gennadios and Weller, 1990; Park et al., 1993; Gontard, et al., 1994; Chinnan and Park, 1995; McHugh et al., 1996).

When active antimicrobial films are used, the antimicrobials contained in the films exert their bactericidal effect on the contacted surface. It was reported that the release of the antimicrobials was dependent on the temperature. Ercolini et al. (2006) stated that low temperature delayed the release of antimicrobials (bacteriocin as the example) from the film by two hours. At room temperature their release was immediate and caused the immediate occurrence of dead cells; however, such a fast release might compromise a long-lasting antimicrobial effect. A decrease in temperature resulted in a reduction in diffusion of potassium sorbate incorporated in K-carrageenan films (Choi et al., 2004) causing less antimicrobial activity. Garcia et al. (2004) reported the reverse results, claiming a more effective action of other antimicrobials (other bacteriocin) at low temperatures. Moreover, Tagg et al. (1976) found that the quantity of antimicrobials that is actually able to exert the bactericidal action could be used to predict the reduction of the population and the probable amount of survivors that may keep growing in the food matrix.

The contact of the cells with the films also determined the antimicrobial effects of the films. Ercolini et al. (2006) found that cells die when they come in contact with the film and cell lysis follows immediately afterwards though depending on the type of antimicrobials. This result indicates that the films reduce the *Listeria* population with efficacy, particularly when the cells died and lysed at the same time, without cell recovery.

### **Carriers**

The characteristics of several polysaccharide materials such as starch and its derivatives, alginates, chitosan and pectinates were reported by Nisperos-Carriedo (1992). In general, due to the hydrophilic nature, high moisture gelatinous polysaccharide coatings retarded moisture loss from food and therefore acted more as sacrificing agents than moisture barriers (Kester and Fennena, 1986).

Native starches and related hydrolyzed products lack active surface and have to be chemically modified or used in conjunction with emulsifying agents in order to encapsulate hydrophobic products. The starch functions as a strong binder for chemical adherence and therefore helps to extend the self life of products. It can be applied to foods as a smooth, glossy and fast drying coating. However, according to Ben Arfa et al. (2007), losses of antimicrobial compounds were always high after the coating and drying process from OSA-starch (a modified starch) coated papers.

Chitosan is a natural polymer obtained by deacetylation of chitin. This white colored powder is insoluble in water while soluble in acidic solvents. Shahidi (1999) reported its intrinsic antimicrobial activity and wide inhibitory efficacy to bacteria due to its positive charged amino groups Liu et al.(2004) hypothesized the interaction between the  $\text{NH}^{4+}$  groups and phosphoryl groups of the phospholipid components of the cell membrane observed by Briandet et al. (1999), probably accounted for chitosan's antimicrobial activity because the positive charge facilitates binding to the membrane. And the killing effect is caused by the structure of the chitosan that allows pore formation in the membrane. Chitosan has proven to be a suitable matrix to form edible coatings and films (Beverly, et al., 2008).

When reacting with calcium ions, alginate will form cold water gels. This non-hazardous odorless hygroscopic powder is water soluble and forms viscous

solutions. Alginate has been chosen as coating carriers in the light of their desirable forming appearances and its consistency in previous studies (Joerger, 2007; Coma, 2008; Hudaa et al., 2010). Moreover, it causes few detectable sensory changes (Hudaa et al., 2010).

Pectin is a water-soluble hygroscopic polymer that is used as a thickening, coating and encapsulating material. It can carry and deliver a variety of bioactive substances (Liu et al., 2006); however, relatively few studies have been reported on the use of pectin coatings (Jin et al., 2009a, b).

Acetic acid is a colorless liquid featured with a pungent odor and a sour taste that could be used to facilitate dissolution of the carrier materials. It is a cheap, and generally recognized as safe (GRAS) substance which serves as an excellent solvent for organic compounds. Acetic acid reduced the numbers of *Listeria monocytogenes* more effectively than lactic acid, citric acid, and hydrochloric acids (Young and Foegeding, 1993; Ita and Hutkins, 1991).

### **Antimicrobials**

The use of edible coating can function as solute, gas and vapor barriers (Gennadios et al., 1997) and serve as effective carriers for a wide range of food additives, including various antimicrobials that can extend RTE food product shelf-life by reducing the risk of pathogen growth on food surfaces (Wong et al., 1996; Cagri et al., 2004; Pranoto et al., 2005), which represent the typical point of entry of pathogens and likely location of maximum microbial contamination (Ming et al., 1997; Janes et al., 2002; Coma, 2008). Incorporating antimicrobial compounds into edible coatings provides a novel way to improve the safety and shelf-life of RTE foods (Cagri et al., 2004). Sodium lactate (SL), is approved for use in fully cooked meat and poultry up to

4.8% (by weight of the total formulation), as a flavoring agent and as a means of inhibiting certain pathogenic bacteria (FDA, 2000). Its antimicrobial activity has been realized by lowering the water activity of foods (Chirife and Fontan, 1980). It is observed that sub-optimum growth temperature (De Wit and Rombouts, 1989) and decreased moisture (Chen and Shelef, 1992) content increased the antimicrobial effects of SL. This aspect of SL played a useful role in controlling the growth of *Listeria monocytogenes* at refrigeration temperatures. 3% SL demonstrated its antilisterial effectiveness on smoked salmon during 40-50 days of storage at 5°C and 10°C (Pelroy et al., 1994). 2% SL at 4°C was able to prevent *Listeria monocytogenes* growth in turkey bologna (Wederquist et al., 1994). A variety of products, such as cooked ground beef (Harmayani et al., 1993), minced beef products (McMahon et al., 1999), sterile comminuted chicken and beef (Shelef and Yang, 1991) were also tested to verify SL's antimicrobial activity.

Sodium diacetate (SD) approved as a flavoring agent in meat and poultry products is capable to prevent the growth of *Listeria monocytogenes* at a level up to 0.25% by weight of the total formulation (FDA, 2000). It completely inhibited growth of *Listeria monocytogenes* in BHI broth at temperatures of 20 and 5°C at a concentration of 0.45% SD (Shelef and Addala, 1994). Inhibition was observed to increase with decreasing temperature. A level of 0.5% SD showed limited inhibition of *Listeria monocytogenes* in turkey slurries and its combination with either SL or ALTA 2341 showed greater inhibition (Schlyter et al., 1993a, b). SD's synergetic antilisterial activity was confirmed by a cooked-in-bag ham study with sodium lactate (Stekelenburg and Kant-Muermans, 2001), showing that additional protection from other antimicrobial would be a better choice to inhibit *Listeria monocytogenes*.

Potassium sorbate (PS) is the GRAS water-soluble salt of sorbic acid. The antimicrobial and preservative properties of sorbic acid have been known since the late 1930's (Luck, 1976, 1980; Sofos and Busta, 1981), and PS is now used widely throughout the world as preservatives for various foods, animal feeds, pharmaceuticals and cosmetics, and for other industrial applications (Sofos, 1989). Generally, sorbates are considered effective food preservatives against yeasts and molds; its activity against bacteria is not as comprehensive and appears to be selective (Sofos, 1989). Effective antimicrobial concentrations of sorbates in most foods are in the range of 0.05 to 0.30. At a level of 0.2%, sorbate was able to inactivate *Listeria monocytogenes* in a broth (El-Shenawy and Marth (1988) and in a cold-pack cheese food (Ryser and Marth, 1988). PS at 1% had limited inhibitory effects on *Listeria monocytogenes* in two commercial cheese brines (Larson et al., 1999). PS treatment made cells of *Listeria monocytogenes* sensitive to high hydrostatic pressure treatment (Mackey et al., 1995; Palou et al., 1997). Combinations of sorbate with propionate or lactate, extended shelf-life and increased safety (Kouassi and Shelef, 1995a,b; Sofos, 2000).

OptiForm<sup>®</sup> PD4 is a commercialised mixture of potassium lactate and SD, which enables reduction of the salt content of up to 40% in cooked meat products without affecting microbial shelf-life. It has good antimicrobial properties and is sold as a formulation of 56% (w/w) L-SL, 40% (w/w) water and 4% (w/w) food grade SD. OptiForm PD4 is completely water soluble. The reduced salt containing OptiForm PD 4 was acceptable from a sensory point of view due to a low concentration of sodium.

Protect-M is a 10-11% (w/w) Na-lauroyl-L-arginine ethyl ester formulation. Protect-M is a self-affirmed GRAS substance and it is dispersible in

water and soluble in ethanol and glycerine. A 0.1-0.2% dilution is recommended for better surface coverage due to the safety sheet.

Guardian™ NR 100 is a synergistic blend of the bacteriocin nisin and rosemary (*Rosmarinus officinalis*) extract, which exhibits antioxidant properties. It is effective in a variety of food products across a wide range of pH levels (3.5-8.0), including low pH processed meat products, chilled, pasteurized ready to eat meals, pasteurized soups and sauces. It is active against gram-positive bacteria, including *Listeria monocytogenes*, extends self life and enhances product quality. The recommended dosage is in the range of 200-500 ppm. Guardian is composed of 75% (w/w) sodium chloride, 4% (w/w) phenolic diterpenes and 1.25% nisin according to the safety sheet provided by the the manufacturer.

NovaGARD™CB1 is another antimicrobial blend. It is a powder composed of maltodextrin, cultured dextrose, SD, sodium chloride, egg white lysozyme and nisin. When used in combination with heat processing, pH, and other formulation adjustments, NovaGARD may delay or prevent outgrowth of selected spore forming and gram positive bacterial strains. Its application areas include deli salads such as chicken, tuna, seafood, ham and ready-to-eat meals. It retards growth of selected gram positive bacteria, protects shelf life by maintaining the intrinsic organoleptic qualities of the finished products, and reduces or eliminates dependence on synthetic preservatives (safety sheets).

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### Chapter 3

## **CONTROL OF *LISTERIA MONOCYTOGENES* ON COLD-SMOKED SALMON AND HAM STEAKS USING CHITOSAN BASED ANTIMICROBIAL COATINGS AND FILMS**

### Abstract

The relatively high incidence of *Listeria monocytogenes* in ready-to-eat (RTE) products such as cold-smoked salmon is of serious concern. The objective of this study was to evaluate the efficacy of chitosan-based edible coatings and films incorporating three Generally Recognized as Safe (GRAS) antimicrobials, sodium lactate (SL), sodium diacetate (SD) and potassium sorbate (PS), against *Listeria monocytogenes* on cold-smoked salmon. Salmon samples were surface-inoculated with a five-strain cocktail of *Listeria monocytogenes* to a final concentration of 4.4 log<sub>10</sub> CFU/cm<sup>2</sup> and then either coated with chitosan solutions or wrapped with chitosan films with or without the three antimicrobials. The samples were then vacuum packaged and stored at 4°C for 30 days. The chitosan coatings with or without the antimicrobials consistently showed higher efficacy against *Listeria monocytogenes* than chitosan films having the same compositions. Chitosan films containing 1.2% SL/0.25% SD or 2.4% SL and chitosan coatings containing 1.2% SL/0.25% SD or 0.15% PS/0.125% SD displayed the greatest antilisterial activity. The effectiveness of chitosan coatings incorporating selected binary antimicrobial combinations (0.15% PS/0.125% SD, 0.15% PS/2.4% SL, 1.2% SL/0.25% SD and 2.4% SL/0.125% SD) against *Listeria monocytogenes* was subsequently tested on ham steaks. The treatment of 1.2% SL/0.25% SD resulted in *Listeria monocytogenes* populations that were 2.3 log<sub>10</sub> CFU/cm<sup>2</sup> lower relative to the control. This study shows that chitosan-based edible coatings and films hold promise and can potentially assist fishery and meat

industries in their efforts to control *Listeria monocytogenes*.

### **Introduction**

*Listeria monocytogenes*, a Gram-positive foodborne pathogen, is a frequent surface contaminant of ready-to-eat (RTE) foods mainly occurring during the post-processing phase (Tompkin, 2002). It has been involved in a number of foodborne illness outbreaks associated with a variety of RTE food products (McLauchlin, 1997). The Centers for Disease Control and Prevention (CDC) have estimated that up to 2,500 cases of listeriosis occur each year in the United States (CDC, 1999, 2000, 2002; Mead, et al., 1999), resulting in 500 deaths. The U.S. Department of Agriculture (USDA-FSIS, 2006) estimated that 15% of all smoked fish is contaminated with *Listeria monocytogenes* (Anonymous, 2001) while Heinitz and Johnson (1998) reported that 17.5% of cold-smoked fish and 8.1% of hot-smoked fish from the United States contained *Listeria monocytogenes*.

Nowadays, consumers demand foods that are safe and possess fresh-like attributes, while modern distribution systems require an adequate shelf-life. The application of antimicrobial packaging, edible films and coatings containing antimicrobials could be an effective means to achieve these goals by controlling the growth of pathogenic and spoilage bacteria. Chitosan is a natural polymer obtained by deacetylation of chitin, which is the major constituent of the exoskeleton of crustaceans (Jeon, 2001). Chitosan is insoluble in water, but soluble in various acidic solvents such as dilute hydrochloric, formic and acetic acids (Shahidi et al., 1999; Ravi Kumar, 2000). Due to its excellent film forming properties, the use of chitosan films and coatings to extend the shelf-life and improve the safety of foods has been widely documented. Jeon et al. (2002) previously reported that chitosan coating

significantly inhibited the growth of microorganisms in fresh fillets of Atlantic cod and herring. Belalia et al. (2008) demonstrated that chitosan coating could be used to control *Listeria monocytogenes* on the surface of RTE roast beef. Zivanovic et al. (2005) applied chitosan films with and without oregano on bologna samples inoculated with *Listeria monocytogenes*. The samples were stored for 5 days at 10°C. Plain chitosan film reduced *Listeria monocytogenes* by 2 log<sub>10</sub> CFU/sample, whereas the films with 1 and 2% oregano decreased the numbers of *Listeria monocytogenes* by 3.6 to 4 log<sub>10</sub> CFU/sample.

In our previous studies (Ye et al., 2008a; b), chitosan was coated onto a piece of plastic film which was then used to sandwich ready-to-eat ham steaks and cold-smoked salmon in order to control the growth of *Listeria monocytogenes*. In both studies, we failed to observe any antilisterial activity of plain chitosan films on either product during long-term refrigerated storage. In the current study, the effect of chitosan applied either as an edible coating or as an edible stand-alone film on the growth of *Listeria monocytogenes* on cold-smoked salmon was further investigated. To further enhance the efficacy of chitosan films and coatings against *Listeria monocytogenes*, three Generally Recognized as Safe (GRAS) antimicrobials, sodium lactate (SL), sodium diacetate (SD) and potassium sorbate (PS), were incorporated into the chitosan films and coatings in this study. These organic salts were chosen as their antilisterial activity has been widely documented elsewhere (El-Shenawy and Marth, 1988; Schlyter et al., 1993; Sofos and Busta, 1993; Szabo and Cahill, 1999; Glass et al., 2002; Islam et al., 2002; Samelis et al., 2002; Lu et al., 2005).

The overall objective of this study was thus to evaluate the efficacy of chitosan-based edible films and coatings incorporating antimicrobials in inhibiting the

growth of *Listeria monocytogenes* on cold-smoked salmon and ham steaks during refrigerated storage.

## **Materials and methods**

### **Preparation of chitosan-based edible coatings and films**

Two grams of low molecular weight chitosan (Sigma-Aldrich, St. Louis, MO) were dissolved in 100 ml of 1% (w/v) acetic acid (Fisher Scientific, NJ) and stirred overnight at room temperature (chitosan concentration of 0.02 g/ml or 2% and pH of 4.51). SL (Purac America Inc., IL), SD (Purac) and PS (Fisher) were dissolved into the chitosan solution singly or in binary combinations to achieve final concentrations shown in Table 3.1. Chitosan films were made by pouring 10 ml of chitosan solutions with or without antimicrobials onto individual petri dishes. The dishes were left uncovered and allowed to dry overnight at 35°C in an incubator. Films were then peeled off the following day, using a sterile tweezer and in close proximity to a Bunsen burner.

### **Inoculum preparation for CSS samples**

A cocktail of five strains of *Listeria monocytogenes*, PSU1 (serotype 1/2a, highly resistant to nisin), F5069 (serotype 4b, highly resistant to chitosan), ATCC19115 (serotype 4b), PSU9 (serotype 1/2b) and Scott A (serotype 4b), were used. All strains were obtained from the culture collections of the University of Delaware and maintained on tryptic soy agar plus 0.6% yeast extract (TSAYE) (Difco Laboratories, Detroit, MI) plates at 4°C. The cultures were transferred monthly onto a freshly made TSAYE agar plate during the experimental period. For growth, a single colony of *Listeria monocytogenes* was inoculated into a tube of tryptic soy broth plus

0.6% yeast extract (TSBYE) (Difco) broth and incubated at 35°C for 24 h. The culture was then transferred to 10 ml of fresh TSBYE and incubated aerobically with agitation for 24 h at 35°C to reach a final concentration of approximately  $10^9$  CFU/ml. A 1-ml volume of each culture was pooled to provide a composite. The cocktail was then serially diluted in 0.1% peptone water (Fisher) to cell densities of ca.  $10^5$  CFU/ml, which served as the inoculums.

### **Samples preparation**

Freshly processed cold-smoked salmon samples were obtained from a producer. They were stored in the freezer at -20°C and brought out to thaw at  $2 \pm 2^\circ\text{C}$  ( $< 4^\circ\text{C}$ ) for 1 day immediately before use as described by Besse et al. (2004). Slices of smoked salmon were punched aseptically into 5.7-cm diameter round pieces weighing  $10 \pm 1$  g with a surface area of  $25.7 \text{ cm}^2$  on each side. The salmon discs were then placed onto a piece of sterile aluminum foil and 125  $\mu\text{l}$  of the five-strain cocktail was spread on one side of the salmon surface, and the samples were left undisturbed for 5 min to allow the inoculum to soak in. Salmon discs were then flipped and inoculated on the other side. The inoculation level was approximately  $4 \log_{10}$  CFU/cm<sup>2</sup>. After inoculation, salmon samples were kept in the refrigerator at 4°C for 20 min to allow bacterial attachment. The inoculated salmon samples were then either coated with chitosan solutions or wrapped with chitosan films prepared above. For coating treatments, 300  $\mu\text{l}$  of the coating solutions was applied and spread evenly on each side of the inoculated samples using a sterile hockey stick. Samples were then allowed to dry by leaving them in a laminar-flow hood under ventilation for about 20 min after the coating was applied on each side for a total drying time of 45 min. For the film treatment, the smoked salmon samples were sandwiched between two chitosan films.

A control, inoculated sample without coating and film, was also prepared. All the samples (the untreated control, coated samples and samples wrapped with films) were then inserted into 3-mil thick high barrier pouches (nylon/polyethylene, Koch Supplies, Kansas City, MO) and subsequently sealed using a vacuum-packaging machine (Model Ultravac 225 with digital control panel, Koch Equipment, Kansas City, MO). The samples were stored at 4°C for 30 days and the populations of *Listeria monocytogenes* in the samples were determined every 6 days.

### **Microbiological analysis of inoculated samples**

For determination of *Listeria monocytogenes* counts, pouches of samples were opened aseptically and the smoked salmon samples were transferred to a sterile stomacher bag and homogenized for 2 min with 40 ml of 0.1% peptone water. Ten-fold serial dilutions were made using 0.1% peptone water. Counts of *Listeria monocytogenes* were determined by an overlay method to enhance recovery of injured cells (Kang and Fung, 1999). Briefly, the serial dilutions were spread-plated on solidified TSAYE agar plates and the plates were incubated at 35°C for 3 h. Approximately 7 ml of modified Oxford medium (Difco) at 45°C was overlaid on the TSAYE plates. The plates were incubated at 35°C and small black colonies with black haloes on the plates were counted after 48 h (Farber and Peterkin, 1991).

### **Inoculation of ham steaks samples**

Freshly processed ham steaks samples were obtained from a local retailer. They were stored in the freezer at -20° C and thawed at 2° C for 1 day immediately before use. Slices of ham steaks were punched aseptically into 5.7-cm diameter round pieces weighing 26.71 g. The ham steaks discs were inoculated with *Listeria*

*monocytogenes* and coated with chitosan solutions with or without antimicrobials. The antimicrobial coating solutions were applied directly onto each surface of the ham steaks samples at 300  $\mu\text{l}$  (to achieve the same final weight/surface concentration,  $\text{g}/\text{cm}^2$ , as cold-smoked salmon) or at 800  $\mu\text{l}$  (to achieve the same final weight/weight concentration,  $\text{g}/\text{g}$ , as cold-smoked salmon). The different antimicrobial treatments were shown in Table 3.2. The samples were dried, vacuum packaged and stored at  $4^\circ\text{C}$  for 12 weeks. The populations of *L. monocytogenes* were determined once a week as described previously.

### **Statistical analysis**

All experiments were replicated three times. Where appropriate, statistical analyses were conducted using JMP<sup>®</sup> 8.0.1 (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) and Tukey's HSD (Honestly Significant Difference) one-way multiple comparisons were used to determine differences in the populations of *Listeria monocytogenes*. Significant differences were considered at the 95% confidence level ( $P < 0.05$ ).

### **Results and discussion**

Table 3.3 shows the effect of chitosan-based edible films and coatings in inhibiting the growth of *Listeria monocytogenes* on cold-smoked salmon during refrigerated storage. The populations of *Listeria monocytogenes* in the control or untreated sample grew steadily reaching  $6.9 \log_{10} \text{CFU}/\text{cm}^2$  by the end of the storage duration. Samples receiving the different surface-treatments consistently produced lower counts of *Listeria monocytogenes* throughout the storage period, primarily due to the inhibitory effect of chitosan and/or the antimicrobials. Plain chitosan film was

able to substantially slow down the growth of *Listeria monocytogenes* since the count of *Listeria monocytogenes* in the plain chitosan film sample at the end of the storage was only  $0.5 \log_{10}$  CFU/cm<sup>2</sup> higher than the initial inoculation level. Comparison of the efficacy of the plain chitosan film against the antimicrobial-containing chitosan films showed that the difference in the counts of *Listeria monocytogenes* in the samples on each sampling day was not significantly different ( $P > 0.05$ ). However, the counts of *Listeria monocytogenes* for samples wrapped with the plain chitosan films were consistently higher than those for the antimicrobial chitosan films, especially towards the end of the storage. Overall, chitosan films incorporating 1.2% SL/0.25% SD or 2.4% SL appeared to be the most effective, reducing the counts *Listeria monocytogenes* by  $> 1 \log_{10}$  after 30 days of storage.

Plain chitosan coating was able to inhibit the growth of *Listeria monocytogenes* during the 30 days of storage. The count of *Listeria monocytogenes* in the sample receiving the plain chitosan coating was  $0.9 \log_{10}$  CFU/cm<sup>2</sup> lower than the initial inoculation level at the end of storage. Incorporation of 1.2% SL/0.25% SD or 0.15% PS/0.125% SD into the chitosan coating furthest enhanced this inhibitory effect; achieving significantly lower counts than samples coated with plain chitosan solution at the end of the 30-day storage ( $P < 0.05$ ).

The chitosan coatings with or without antimicrobials consistently showed higher efficacy than chitosan films having the same compositions. The average difference in *Listeria monocytogenes* counts for all the treatments between the coatings and films was  $1.4 \log_{10}$  CFU/cm<sup>2</sup>; with a maximum significant difference of  $2.2 \log_{10}$  CFU/cm<sup>2</sup> for the treatment of 1.2% SL/0.3% PS on day 30 ( $P < 0.05$ ). Comparison of the concentrations of chitosan in the films and coatings showed that

films (4.29 mg/cm<sup>2</sup>) had a higher concentration of chitosan than coatings (0.233 mg/cm<sup>2</sup>). The fact that samples treated with coatings produced lower counts shows that the inhibitory effect was to some extent due to the nature of the coatings themselves and in the way in which they were applied onto the smoked salmon samples. Chitosan coatings were prepared and directly applied onto smoked salmon, whereas chitosan films were applied onto the samples after being formed separately. Unlike films, the aqueous coating solutions would be expected to contact more effectively the surfaces and pores of the smoked salmon, thought to be likely hiding places for *Listeria monocytogenes*. Moreover, the coatings were also able to permeate into the core of the product, thus acting as a carrier for antimicrobials to access microenvironments of the smoked salmon slices where the bacterial inoculum could have also penetrated. Chitosan is also known to display intrinsic antimicrobial activity which is more effectively expressed in aqueous systems (Sudarshan et al., 1992; Wang, 1992). Ouattara et al. (2000) stated that the antimicrobial activity of chitosan is closely linked with its ability to disperse within food products. Since chitosan entrapped in films is in a less dissoluble form, it would be anticipated that the movement of chitosan molecules as well as the incorporated antimicrobials would be more restricted with an accompanying decrease in their antimicrobial activity (Liu, et al, 2001). Although chitosan was dissolved in 1% acetic acid, we do not anticipate that acetic acid would present a major contribution to the antimicrobial effect of the coatings. Neetoo et al. (2010) previously studied the efficacy of alginate dissolved in 1% acetic acid to control the development of *Listeria monocytogenes* on cold-smoked salmon. They demonstrated minimal differences in the counts of *Listeria monocytogenes* between untreated samples and those treated with the plain alginate coating during a

30-day storage at refrigeration temperature. This shows therefore that the presence of the solvent had minimal antimicrobial effect.

Compared to chitosan, the antimicrobials incorporated into the coating matrix were salts of organic acids that have a relatively smaller size and molecular weight. These small molecules can more readily disperse into a food matrix. However, the advantage of entrapping them into a hydrocolloid solution or film is that the diffusion is more retarded thereby maintaining a higher local concentration of the antimicrobials on the surface for a longer period of time. Vojdani and Torres (1989) showed that PS incorporated into edible films and subsequently applied onto the surface of food was able to move at a controllable rate into the samples. Chitosan film or coating containing 1.2% SL/0.25% SD was one of the two most effective antimicrobial combinations against *Listeria monocytogenes* and this is supportive of the findings of Blom et al. (1997) who demonstrated that a mixture of 2.5% SL and 0.25% acetate were capable of preventing the growth of *Listeria monocytogenes* in servelat sausage while maintaining the sensory acceptability of the sausage. This synergistic inhibitory effect on the growth of *Listeria monocytogenes* was also in agreement with findings of Stekelenburg and Kant-Muermans (2001) who showed that *Listeria monocytogenes* was inhibited by 2.5 or 3.3% SL in combination with 0.2% SD on vacuum-packaged cooked ham. In addition, the synergistic effect of SL and SD on inhibiting the growth of *Listeria monocytogenes* was observed in cold-smoked salmon slices and pâté (Neetoo et al., 2008), frankfurters (Samelis et al., 2002), turkey products (Schlyter et al., 1993), wieners (Glass et al., 2002), and beef bologna (Mbandi and Shelef, 2002).

It is worth noting that the populations of *Listeria monocytogenes* in

samples treated with 1.2% SL/0.30% PS film were higher than those in samples treated with 1.2% SL/0.15% PS film although the differences in the counts of *Listeria monocytogenes* on each sampling day was not significant ( $P > 0.05$ ). We observed that the chitosan films incorporating 1.2% SL/0.30% PS resulted in the formation of crystals after the coating solution was cast into petri dishes and allowed to dry. The appearance of crystals on the film was likely due to the crystallization of PS and/or SL which was caused by the reduced solubility of the salts as the solvent (water and acetic acid) evaporated. As a result, we speculate that this precipitation effect probably reduced the diffusion rate of the salts into the salmon thereby decreasing its overall antimicrobial effect. Chitosan films incorporating 1.2% SL/0.15% PS and other antimicrobials were not observed to form any crystals during drying.

Several authors have already shown that the addition of antimicrobials such as SL to ham (Zhu et al., 2005) or SL in combination with SD to cold smoked salmon (Vogel et al., 2006) brought about minimal impact on the sensory quality of the product. Jo et al. (2001) did not observe any difference in color, flavor, texture and overall acceptance in the quality of pork sausage incorporating a water-soluble chitosan oligomer (0.2%). Neetoo et al. (2010) also did not observe any alterations in the color, texture and overall commercial appeal of smoked salmon product treated with an alginate-based coating incorporating similar antimicrobials. Similarly, we observed that un-inoculated cold-smoked salmon samples treated with chitosan coatings and films did not show any abnormal changes in coloration or aroma during the entire storage period. We thus anticipate that the application of chitosan incorporating organic salts would present minimal sensory concern.

Fig. 3.1 shows the effect of chitosan-based edible coatings containing low

level GRAS antimicrobials (same weight/surface concentration, g/cm<sup>2</sup>, as cold-smoked salmon) on the growth of *L. monocytogenes* on ham steaks. The average standard deviation for all the data points in this figure was 0.27 log<sub>10</sub> CFU/cm<sup>2</sup>. The initial concentration of *L. monocytogenes* on inoculated ham steaks samples was 3.2 log<sub>10</sub> CFU/cm<sup>2</sup>. *L. monocytogenes* in the control sample grew to 4 log<sub>10</sub> CFU/cm<sup>2</sup> after 12 weeks of storage at refrigerated temperature. Unlike cold-smoked salmon, the population of *L. monocytogenes* in the control ham steaks sample did not support a steady growth of the inoculum. This was probably because the pre-packaged ham steaks purchased from the grocery store already contained salts of organic acids which are used widely in food industry as preservatives, thus accounting for the slower growth compared to cold-smoked salmon. Incorporating antimicrobials into chitosan significantly inhibited the growth of *L. monocytogenes* (P<0.05). The treatments of 0.89% SL/0.047% SD and 0.056% PS/0.89% SL were the most effective and resulted in counts of *L. monocytogenes* that were significantly lower than those in the control sample (P<0.05) at the end of storage. The counts of *L. monocytogenes* in the other two antimicrobial treatments, 0.44% SL/0.093% SD and 0.056% PS/0.047% SD, were not significantly different from those of the control sample (P>0.05). On average, the four treatments reduced the initial counts from 3.2 log<sub>10</sub> CFU/cm<sup>2</sup> to 2.8 log<sub>10</sub> CFU/cm<sup>2</sup> by the end of the 12-week storage.

Fig. 3.2 shows the effect of chitosan-based edible coatings containing high levels of antimicrobials (same weight/weight concentration, g/g, as cold-smoked salmon) on the growth of *L. monocytogenes* on ham steaks during refrigerated storage. The average standard deviation for all the data points in this figure was 0.25 log<sub>10</sub> CFU/cm<sup>2</sup>. On average, the treatments reduced the initial counts to 2.1 log<sub>10</sub> CFU/cm<sup>2</sup>

amounting to  $\sim 1 \log_{10}$  CFU/cm<sup>2</sup> reduction. Among all the treatments, 1.2% SL/0.25% SD was significantly more effective than the other three treatments, reducing the initial counts from 3.2 to 1.7  $\log_{10}$  CFU/cm<sup>2</sup> after 12-week of storage ( $P < 0.05$ ). The count of *L. monocytogenes* at the end of storage in this treatment was around 0.5  $\log_{10}$  CFU/cm<sup>2</sup> lower than the other three treatments. The plain chitosan coating in this part of the study had a higher chitosan concentration (0.623 mg/cm<sup>2</sup> or 1.20 mg/g) than the one used in Fig. 3 (0.233 mg/cm<sup>2</sup> or 0.45 mg/g), which could explain the slightly higher antimicrobial effect of chitosan coatings in Fig. 3.2 compared to Fig. 3.1.

The application of plain chitosan coating at a concentration of 1.20 mg/g was able to inhibit and even reduce the populations of *L. monocytogenes* on both cold-smoked salmon and ham steaks during long term refrigerated storage. Incorporation of antimicrobials further enhanced the antilisterial effect. Overall, chitosan-based coatings incorporating 1.2% SL and 0.25% SD consistently delivered the most effective antilisterial activity. This synergistic inhibitory effect on the growth of *L. monocytogenes* was in line with findings by Stekelenburg and Kant-Muermans (2001) who showed that *L. monocytogenes* was inhibited by 2.5 or 3.3% SL in combination with 0.2% SD on vacuum-packaged cooked ham. In addition, synergistic effect of SL and SD on inhibition of the growth of *L. monocytogenes* was observed in frankfurters (Samelis et al., 2002), turkey products (Schlyter et al., 1993), wieners (Glass et al., 2002), and beef bologna (Mbandi and Shelef, 2002). Several authors have shown that the addition of SL to ready-to-eat products brought about minimal impact on the sensory quality (Zhu et al., 2005). Jo et al. (2001) did not observe any difference in color, flavor, texture, overall acceptance, and mechanical texture in the quality of pork sausage incorporated with water-soluble chitosan oligomer (0.2%). We thus anticipate

that the application of chitosan incorporating organic salts would present minimal sensory concern.

## **CONCLUSION**

In this study, the effect of chitosan-based edible films and coatings incorporating GRAS antimicrobials against the growth of *Listeria monocytogenes* was investigated in cold-smoked salmon during a 30-day refrigerated storage. The result showed that the chitosan coatings with or without the antimicrobials consistently showed higher efficacy against *Listeria monocytogenes* than chitosan films having the same compositions and plain chitosan coating without antimicrobials was able to inhibit the populations of *Listeria monocytogenes* on cold-smoked salmon during refrigerated storage. Among all the treatments, chitosan films containing 1.2% SL/0.25% SD or 2.4% SL and chitosan coatings containing 1.2% SL/0.25% SD or 0.15% PS/0.125% SD displayed the greatest antilisterial activity. Our research thus holds great promise to enhance the microbiological safety of RTE fishery products.

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**FIGURES**

**Figure 3.1**

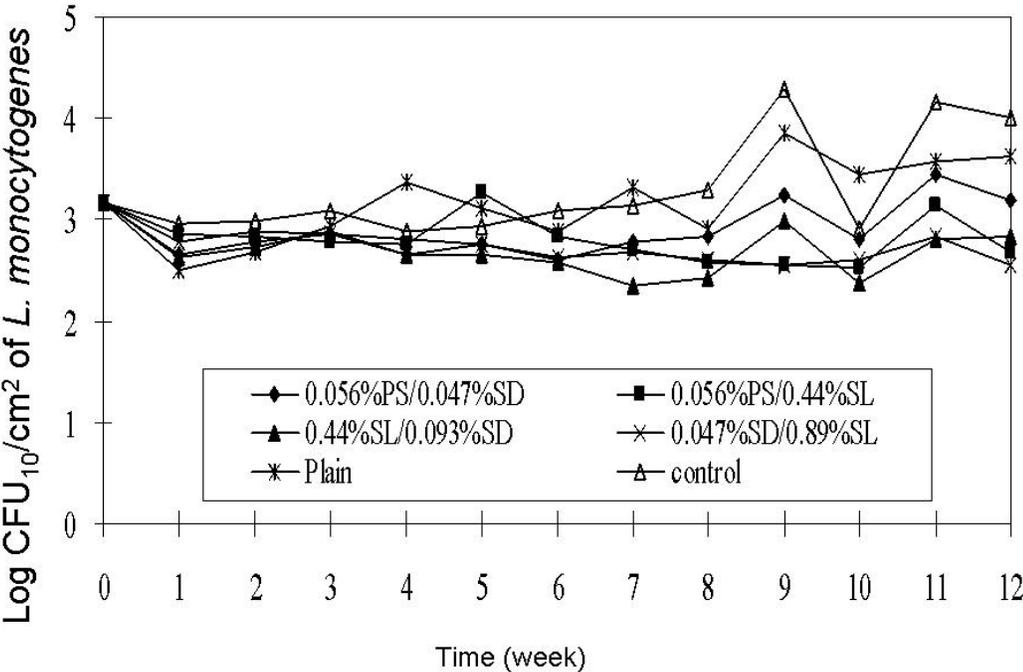
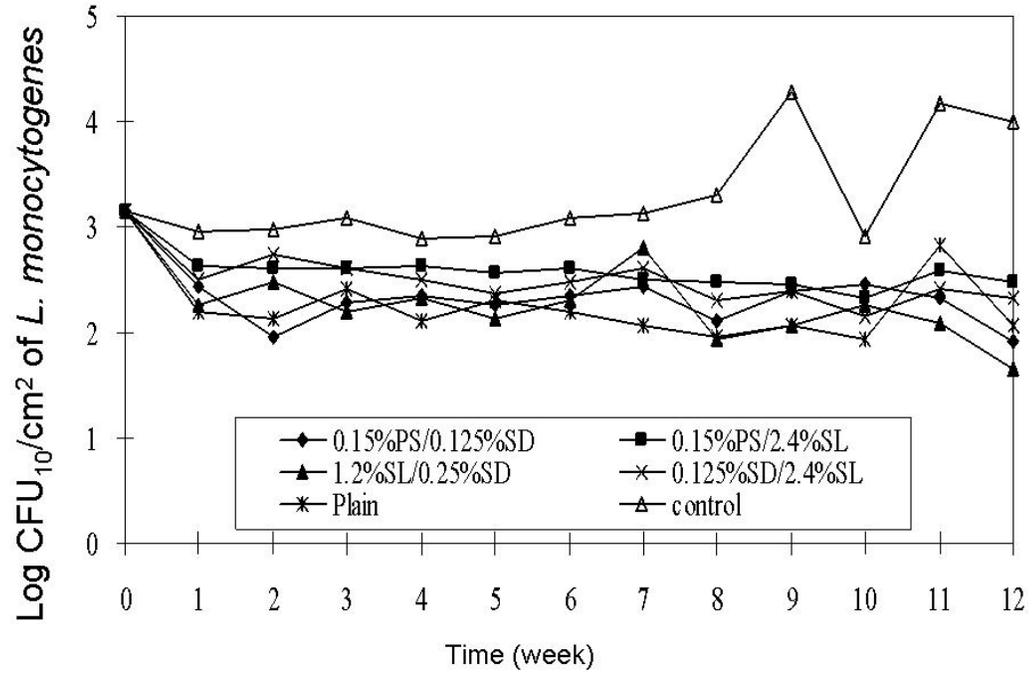


Figure 3.2



## **TABLES**

**Table 3.1**

Antimicrobial concentrations (%weight of antimicrobials/weight of smoked salmon) in chitosan coatings and films applied onto cold-smoked salmon. The concentrations were chosen on the basis of the legal limits. And binary combinations were consisted by a low level of an antimicrobial plus a high level of another antimicrobial or low levels of two antimicrobials.

SL	SD	PS
0	0	0.3
2.4	0	0
0	0.25	0
1.2	0	0.15
0	0.125	0.15
1.2	0.125	0
2.4	0	0.15
0	0.25	0.15
1.2	0	0.3
1.2	0.25	0
0	0.125	0.3
2.4	0.125	0
0	0	0

The SL concentrations refer to pure SL.

**Table 3.2**

Antimicrobial concentrations (%weight of antimicrobials/weight of ham steaks) in chitosan coatings applied onto ham steaks.

Low Concentrations			High Concentrations		
SL	SD	PS	SL	SD	PS
0	0.047	0.056	0	0.125	0.15
0.89	0	0.056	2.4	0	0.15
0.89	0.047	0	2.4	0.125	0
0.44	0.093	0	1.2	0.25	0
0	0	0	0	0	0

**Table 3.3**

Effect of chitosan-based edible films and coatings incorporating GRAS antimicrobials on the growth of *Listeria monocytogenes* on vacuum packaged cold-smoked salmon stored at 4°C for 30 days. The initial inoculation level was 4.4 log<sub>10</sub> CFU/cm<sup>2</sup>. Data are the means of three replicates ± one standard deviation (log<sub>10</sub> CFU/cm<sup>2</sup>).

Treatment	6 days		12 days		18 days		24 days		30 days	
	Coating	Film	Coating	Film	Coating	Film	Coating	Film	Coating	Film
Control	5.0±0.3 <sup>aA</sup>	5.2±0.2 <sup>aA</sup>	6.1±0.3 <sup>aA</sup>	6.1±0.3 <sup>aA</sup>	6.4±0.6 <sup>aA</sup>	6.4±0.6 <sup>aA</sup>	6.6±0.04 <sup>aA</sup>	6.6±0.04 <sup>aA</sup>	6.9±0.7 <sup>aA</sup>	6.9±0.7 <sup>aA</sup>
Plain	2.5±0.5 <sup>bA</sup>	3.1±0.7 <sup>bA</sup>	3.5±0.6 <sup>bA</sup>	3.4±0.3 <sup>bcA</sup>	2.7±0.6 <sup>bA</sup>	3.6±0.5 <sup>bA</sup>	3.2±0.9 <sup>bA</sup>	4.1±0.7 <sup>bA</sup>	3.5±0.7 <sup>bA</sup>	4.9±0.9 <sup>abA</sup>
0.3% PS	2.3±0.6 <sup>bA</sup>	2.9±0.3 <sup>bA</sup>	3.0±0.3 <sup>bA</sup>	2.8±0.7 <sup>bcA</sup>	2.7±1.1 <sup>bA</sup>	2.7±0.1 <sup>bA</sup>	2.8±0.6 <sup>bcA</sup>	3.6±0.9 <sup>bA</sup>	2.8±1.3 <sup>bcA</sup>	3.7±1.2 <sup>bA</sup>
2.4% SL	2.5±0.7 <sup>bA</sup>	3.3±0.7 <sup>bA</sup>	2.7±0.5 <sup>bA</sup>	3.4±0.3 <sup>bcA</sup>	2.7±0.3 <sup>bA</sup>	2.2±0.7 <sup>bA</sup>	2.5±0.6 <sup>bcA</sup>	2.8±0.04 <sup>bA</sup>	2.7±0.6 <sup>bcA</sup>	3.0±0.3 <sup>bA</sup>
1.2% SL/0.125% SD	2.3±0.1 <sup>b</sup>	ND	2.6±0.6 <sup>b</sup>	ND	2.8±0.3 <sup>b</sup>	ND	2.4±0.4 <sup>bc</sup>	ND	2.7±0.2 <sup>bc</sup>	ND
0.25% SD	2.3±0.6 <sup>bA</sup>	2.6±0.2 <sup>bA</sup>	2.3±0.5 <sup>bA</sup>	3.1±0.3 <sup>bcA</sup>	2.4±0.4 <sup>bA</sup>	3.0±0.3 <sup>bA</sup>	2.7±0.2 <sup>bcA</sup>	3.5±0.6 <sup>bA</sup>	2.5±0.3 <sup>bcA</sup>	3.5±0.2 <sup>bB</sup>
0.15% PS/1.2% SL	1.9±0.3 <sup>bA</sup>	2.8±0.3 <sup>bB</sup>	2.7±0.5 <sup>bA</sup>	4.3±1.3 <sup>bA</sup>	2.7±0.2 <sup>bA</sup>	2.2±0.5 <sup>bA</sup>	2.8±0.5 <sup>bcA</sup>	3.5±0.6 <sup>bA</sup>	2.4±0.7 <sup>bcA</sup>	3.3±1.0 <sup>bA</sup>
1.2% SL/0.3% PS	2.7±0.6 <sup>bA</sup>	3.6±0.8 <sup>abA</sup>	2.8±0.4 <sup>bA</sup>	3.7±0.8 <sup>bcA</sup>	2.9±0.8 <sup>bA</sup>	3.7±1.2 <sup>bA</sup>	2.9±0.5 <sup>bcA</sup>	3.6±1.3 <sup>bA</sup>	2.4±0.4 <sup>bcA</sup>	4.6±0.5 <sup>abB</sup>
0.15% PS/0.25% SD	2.5±0.4 <sup>bA</sup>	2.8±0.2 <sup>bA</sup>	2.6±0.6 <sup>bA</sup>	2.9±0.3 <sup>bcA</sup>	1.8±0.1 <sup>bA</sup>	3.1±0.3 <sup>bB</sup>	1.6±0.4 <sup>cA</sup>	3.8±0.6 <sup>bB</sup>	2.4±0.5 <sup>bcA</sup>	3.7±0.04 <sup>bB</sup>
0.125% SD /0.3% PS	2.3±0.4 <sup>bA</sup>	3.0±0.6 <sup>bA</sup>	2.3±0.5 <sup>bA</sup>	3.1±0.6 <sup>bcA</sup>	2.0±0.5 <sup>bA</sup>	2.7±0.4 <sup>bA</sup>	2.1±0.6 <sup>bcA</sup>	2.8±0.2 <sup>bA</sup>	2.1±0.6 <sup>bcA</sup>	3.8±1.9 <sup>bA</sup>
0.15% PS/2.4% SL	2.8±0.5 <sup>bA</sup>	3.1±1.1 <sup>bA</sup>	2.3±0.8 <sup>bA</sup>	3.2±0.6 <sup>bcA</sup>	2.7±1.3 <sup>bA</sup>	2.8±0.7 <sup>bA</sup>	2.3±0.2 <sup>bcA</sup>	3.2±1.3 <sup>bA</sup>	1.7±0.5 <sup>bcA</sup>	3.4±1.2 <sup>bA</sup>
0.125% SD /2.4% SL	2.7±0.4 <sup>bA</sup>	2.9±0.5 <sup>bA</sup>	2.7±0.03 <sup>bcA</sup>	2.9±0.4 <sup>bcA</sup>	2.0±0.2 <sup>bA</sup>	2.4±0.4 <sup>bA</sup>	2.0±0.1 <sup>bcA</sup>	2.6±0.03 <sup>bB</sup>	1.7±0.05 <sup>bcA</sup>	3.5±0.5 <sup>bB</sup>
0.15% PS/0.125% SD	1.8±0.1 <sup>b</sup>	ND	2.2±0.5 <sup>b</sup>	ND	2.2±0.6 <sup>b</sup>	ND	2.2±0.8 <sup>bc</sup>	ND	1.6±0.9 <sup>c</sup>	ND
1.2% SL/ 0.25% SD	2.6±0.2 <sup>bA</sup>	2.4±0.4 <sup>bA</sup>	2.8±0.5 <sup>bA</sup>	2.6±0.2 <sup>cA</sup>	2.2±0.5 <sup>bA</sup>	3.1±0.4 <sup>bB</sup>	1.7±0.2 <sup>cA</sup>	2.7±0.4 <sup>bB</sup>	1.4±0.3 <sup>cA</sup>	3.1±0.7 <sup>bB</sup>

ND = not done because these two treatments could not form edible films with desirable physical characteristics.

Values in the same column followed by the same lower-case letter are not significantly different (P > 0.05).

On each sampling day, values in the same row followed by the same upper-case letter are not significantly different (P > 0.05).

## Chapter 4

### **CONTROL OF *LISTERIA MONOCYTOGENES* ON ROASTED TURKEY USING ANTIMICROBIAL EDIBLE COATINGS DURING FREEZING AND/OR REFRIGERATED STORAGE**

#### **Abstract**

The growth of *Listeria monocytogenes* on processed meat products such as roasted turkey has become a major concern and an important food safety issue. The meat industry has incorporated hurdles such as the application of antimicrobials and freezing temperature to prevent *Listeria monocytogenes*. The objective of the first phase of this study was to evaluate the efficacy of four polysaccharide-based edible coatings (starch, chitosan, alginate and pectin) incorporating six Generally Recognized as Safe (GRAS) antimicrobials, sodium lactate (SL), sodium diacetate (SD), OptiForm<sup>®</sup> PD4, NovaGARD<sup>™</sup>CB1, Protect M and Guardian<sup>™</sup> NR 100 against *Listeria monocytogenes* on roasted turkey. Roasted turkey slices were surface-inoculated with a five-strain cocktail of *Listeria monocytogenes* to a final concentration of  $\sim 3 \log_{10}$  CFU/cm<sup>2</sup> and coated with the edible coatings with or without antimicrobials. The samples were vacuum packaged and stored at 4°C for up to 8 weeks. The pectin coatings incorporating selected antimicrobials were the best treatment group with higher efficacy against *Listeria monocytogenes* than other coatings. Therefore, in the second phase of the study, we investigated the sequential application of frozen storage and refrigeration to further delay to the growth of *Listeria monocytogenes*. Following 4 weeks of frozen storage and 8 weeks of

refrigeration, antimicrobial-containing pectin coatings significantly lowered *Listeria monocytogenes* populations, with 2.5% OptiForm displaying the greatest antilisterial activity (1.2 log<sub>10</sub> CFU/cm<sup>2</sup> reduction). Although freezing duration prior to refrigeration did not have a significant influence on the *Listeria monocytogenes* population, frozen storage lasting four weeks reduced the counts of *Listeria monocytogenes* to a greater extent (P > 0.05). Growth of aerobic and anaerobic microbes was generally inhibited using pectin coatings incorporating antimicrobials after a 4-week frozen and 8-week refrigerated storage. This study shows that pectin-based antimicrobial edible coatings hold promise in enhancing the safety of ready-to-eat turkey products, and frozen storage has the potential to enhance their effectiveness.

### **Introduction**

*Listeria monocytogenes* is a gram-positive, non-sporeforming pathogenic bacterium that can result in foodborne bacterial infections with symptoms ranging from a mild non-invasive listerial gastroenteritis to severe and sometimes life-threatening invasive listeriosis (CFSAN, 2003; CFSAN, 2008). The Center of Disease and Prevention estimates that there are about 2500 cases of illnesses and 500 deaths due to listeriosis each year (CFSAN, 2003), making it a significant public health concern. Multiple multistate outbreaks associated with RTE meat products such as deli meat and hotdogs have occurred (CDC, 1999; 2000; 2002). Potential sources of *Listeria monocytogenes* in foods include contaminated raw materials, processing aids, and processing equipment that cross-contaminate food (Tompkin et al., 1999; USFDA/FSIS, 2003).

Various measures have been investigated to control *Listeria monocytogenes* in food (NACMC, 1991; Tompkin et al., 1999; Tompkin, 2002;

Moretro and Lansgrund, 2004; Gall et al., 2004; Kornacki, 2005; Scott et al., 2005). Freezing is one hurdle known to inhibit *Listeria monocytogenes* (Sorrells et al., 1989; Tienungoon et al., 2000; IFT/FDA, 2001) by significantly slowing down the growth (Lou and Yousef, 1999; IFT/FDA, 2001; USFDA/FSIS, 2003). Guldager et al. (1998) and Lund (2000) showed that frozen storage has the potential to inhibit or inactivate both spoilage and pathogenic micro-organisms while previous studies by Dalgaard and Jørgensen (2000) revealed that freezing and its combination with brining were common preservation ways to extend the short saleable life of raw shrimps.

The use of antimicrobial edible coating has also been shown to delay growth of *Listeria monocytogenes* on various foods (Gadang, 2008; Martins, 2010; Neetoo et al., 2010). The use and functionalities of various polysaccharide materials such as starch and its derivatives, alginates, chitosan and pectinates in food were previously reported by Nisperos-Carriedo (1994). Pregelatinized starch (modified corn starch) can readily hydrate in both cold and hot water, and is a strong binder for chemicals, thus helping to extend the shelf life of products. Chitosan is a natural polymer obtained by the deacetylation of chitin that dissolves in acid to produce a solution with a desirable viscosity and a wide antibacterial spectrum (Shahidi, 1999). Alginate forms cold water gels while pectin is a water-soluble polymer that can also act as an effective delivery system for a variety of bioactive substances (Liu et al., 2006). Starch, alginate and chitosan as edible coating materials have been widely studied (Kim, S.H. et al., 2009; Zhu, X et al., 2008; Vinitnantharat, S., et al., 2007; Ben Arfa, A, et al., 2007; Datta, S., et al., 2008) while the use of pectin as an edible polymer on RTE meat products has been investigated to a lesser extent (Jin, T., et al., 2009a).

In our study, we incorporated various antimicrobials into each of the aforementioned polymers to compare their antilisterial efficacy. OptiForm<sup>®</sup> PD4 consists of 56% (w/w) L-potassium lactate, 40% (w/w) water and 4% (w/w) food grade sodium diacetate (SD) and is completely water soluble. Protect-M is a formulation consisting of 10-11% (w/w) lauric arginate, a novel antimicrobial compound derived from lauric acid, arginine and ethanol that is dispersible in water. Guardian<sup>™</sup> NR 100 is a natural synergistic blend of the bacteriocin nisin and natural rosemary (*Rosmarinus officinalis*) extract. It is bacteriostatic towards various gram-positive pathogenic and spoilage organisms, thus helping to enhance the microbiological safety and quality of meat products and chilled, pasteurized “ready to eat meals”. NovaGARD<sup>™</sup>CB1 consists of various active ingredients: egg white lysozyme, a nisin preparation and sodium diacetate. Its application ensures retarded growth of selected gram-positive bacteria as well as extends the shelf life of select refrigerated foods.

The objectives of our study were to (1) compare the efficacy of various antimicrobial coating formulations in inhibiting growth of *Listeria monocytogenes* on roasted turkey during refrigerated storage and to (2) investigate the successive application of frozen storage and refrigeration to enhance the antimicrobial activity of these coating formulations.

### **Materials and Methods**

#### **Antilisterial effectiveness of various types of edible coatings incorporating antimicrobials on roasted turkey during refrigerated storage**

##### **Preparation of edible coatings**

Four types of carbohydrate polymers were used: starch (Grain Processing

Corporation, IA), low molecular weight chitosan (Sigma-Aldrich, St. Louis, MO), alginate (TIC GUMS, MD) and pectin (low methoxyl pectin, standardized with dextrose, TIC GUMS, MD). The coating solutions were prepared by mixing 15 g of starch, 2 g of chitosan, 1 g of alginate and 1 g of pectin in 100 ml of a 1% acetic acid (Fisher Scientific, NJ) with overnight stirring at room temperature (22°C). Sodium lactate (SL, Purac America Inc., IL), SD (Purac), Opti.Form PD4 (Purac), Protect-M (Purac), Guardian NR100 (Danisco, USA Inc, KS) and Nova GARD™ CB1 (Danisco) were added into the coating solutions singly or in binary combinations to achieve the final concentrations shown in Table 4.1.

### **Inoculum preparation**

A cocktail of five strains of *Listeria monocytogenes*, PSU1 (serotype 1/2a, highly resistant to nisin), F5069 (serotype 4b, highly resistant to chitosan), ATCC19115 (serotype 4b), PSU9 (serotype 1/2b) and Scott A (serotype 4b), were used. All strains were from the culture collection at the University of Delaware and stored on tryptic soy agar plus 0.6% yeast extract (TSAYE) (Difco Laboratories, Detroit, MI) agar at 4°C. The cultures were transferred monthly onto a fresh TSAYE plate. For the preparation of inoculums, a single colony of *Listeria monocytogenes* was transferred into a tube of tryptic soy broth plus 0.6% yeast extract (TSBYE) (Difco) broth and incubated at 35°C for 24 h. The culture was then transferred to 10 ml of fresh TSBYE and incubated for 24 h at 35°C to reach a final concentration of approximately 10<sup>9</sup> CFU/ml. A 1-ml volume of each culture was pooled to form a five-strain cocktail. The cocktail was serially diluted in 0.1% peptone water (Fisher) to cell densities of ca. 10<sup>5</sup> CFU/ml, which served as the inoculum.

### **Sample preparation**

Freshly processed roasted turkey samples were obtained from a local store, stored at  $-20^{\circ}\text{C}$  and thawed at  $2 \pm 2^{\circ}\text{C}$  ( $< 4^{\circ}\text{C}$ ) for 1 day before use. Slices of roasted turkey were punched aseptically into 5.7-cm diameter round pieces weighing  $30 \pm 1$  g with a surface area of  $25.7 \text{ cm}^2$  on each side. The roasted turkey discs were placed onto a piece of sterile aluminum foil and  $158 \mu\text{l}$  of the five-strain cocktail was spread on one side of the turkey surface, and the samples were left undisturbed for 5 min. Turkey discs were then flipped and inoculated on the other side. After inoculation, turkey samples were kept in the refrigerator at  $4^{\circ}\text{C}$  for 20 min to allow bacterial attachment. The inoculation level was approximately  $3 \log_{10} \text{ CFU}/\text{cm}^2$ , determined microbiologically once the inoculated samples were ready. Then,  $935 \mu\text{l}$  of the coating solutions was applied and spread evenly on each side of the inoculated samples using a sterile hockey stick. The coating on each side was allowed to dry in a laminar-flow hood under ventilation for about 20 min each. A control, inoculated sample without coating was also prepared. The were inserted into 3-mil thick high barrier pouches (nylon/polyethylene, Koch Supplies, Kansas City, MO) and sealed using a vacuum-packaging machine (Model Ultravac 225 with digital control panel, Koch Equipment, Kansas City, MO). The samples were stored at  $4^{\circ}\text{C}$  for 8 weeks and the populations of *Listeria monocytogenes* in the samples were determined every week.

### **Microbiological analysis of inoculated samples**

For determination of *Listeria monocytogenes* counts, pouches of samples were opened aseptically and the turkey samples were transferred to a sterile stomacher bag and homogenized for 2 min with 100 ml of 0.1% peptone water. Ten-fold serial dilutions were made using 0.1% peptone water. Counts of *Listeria monocytogenes* were determined by an overlay method to enhance recovery of injured cells (Kang and

Fung, 1999). Briefly, the serial dilutions were spread-plated on solidified TSAYE agar plates and the plates were incubated at 35°C for 3 h. Approximately 7 ml of modified Oxford medium (Difco) at 45°C was overlaid on the TSAYE plates. The plates were incubated at 35°C and small black colonies with black haloes on the plates were counted after 48 h (Farber and Peterkin, 1991).

### **Frozen-refrigerated storage study in pectin-based edible coatings on roasted turkey**

SL, SD, Opti.Form PD4, Protect-M, Guardian NR100 and Nova GARD™ CB1 were added into the pectin coating solution singly or in binary combinations (Table 4.2). The turkey discs were prepared, inoculated with *Listeria monocytogenes*, coated with the pectin coating solutions, and air-dried as described above. Controls, untreated samples consisting of inoculated turkey samples without coatings, were also prepared. In addition, un-inoculated turkey samples were subjected to the same treatments. The samples were vacuum packaged and stored in a freezer at  $-18\pm 1^{\circ}\text{C}$  for 4 weeks. For the inoculated samples, five sets each consisting of seven pectin coating treatments and one inoculated control were removed from the freezer every week and thawed at 4°C overnight. One set of the samples was used to determine the counts of *Listeria monocytogenes* immediately after thawing and the remaining sets were stored at 4°C for up to 8 weeks. The populations of *Listeria monocytogenes* in one set of the samples were determined every two weeks. For the un-inoculated samples, one set consisting of seven pectin coating treatments and one control were removed from the freezer every week, thawed at 4°C overnight and stored at 4°C for 8 weeks. The aerobic and anaerobic bacteria counts in the un-inoculated samples were determined after 8 weeks of refrigerated storage. Aerobic bacteria counts were determined by

plating onto TSAYE plates and incubated aerobically at 35°C for 2 days. Anaerobic bacterial counts were determined on Anaerobic Agar (Difco Laboratories) plates incubated in anaerobic jars with Gas Paks (BBL) at 35°C for 2 days.

#### **Statistical analysis.**

All experiments were replicated three times. Where appropriate, statistical analyses were conducted using JMP<sup>®</sup> 8.0.1 (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) and Tukey's HSD (Honestly Significant Difference) one-way multiple comparisons were used to determine differences in the populations of *Listeria monocytogenes*. Significant differences were considered at the 95% confidence level (P<0.05).

### **Results and Discussion**

#### **Effect of four different types of coatings incorporating antimicrobials on the growth of *Listeria monocytogenes* on roasted turkey during refrigerated storage**

Fig. 4.1 shows the effect of the four edible coatings on inhibiting growth of *Listeria monocytogenes* on roasted turkey during refrigerated storage. Error bars were omitted from the figures to allow for easier interpretation. The populations of *Listeria monocytogenes* in the untreated control sample grew steadily reaching approximately 7.0 log<sub>10</sub> CFU/cm<sup>2</sup> at week 4 and remained around that level until the end of 8-week storage. Growth of *Listeria monocytogenes* in plain starch coating and coatings containing 0.1% Protect M or 500 ppm Guardian was very similar to that in the control (Fig. 4.1a). The other starch antimicrobial coating treatments produced counts that were lower than those of the control throughout the entire storage. Starch coatings receiving 0.25% NovaGARD and 2% SL/0.25% SD displayed the greatest antilisterial efficacy with final counts *Listeria monocytogenes* lower than those of

control samples by  $2.1 \log_{10} \text{CFU/cm}^2$  at the end of the storage, although the difference was not statistically significantly ( $P < 0.5$ ).

The chitosan coating treatments were able to slow down the growth of *Listeria monocytogenes* to varying degrees during the 8-week storage. The plain chitosan coating was able to delay the growth of *Listeria monocytogenes*, with a final population of  $2.1 \log_{10} \text{CFU/cm}^2$  lower than that of control ( $P < 0.05$ ) (Fig. 4.1b). The effectiveness of the treatments were in the order of 2.5% OptiForm PD4/0.1% Protect M > 0.1% Protect M > plain chitosan > 500 ppm Guardian NR100 > 2.5% Optiform > 0.25% SD. At the end of storage, the *Listeria monocytogenes* populations for these 6 treatments were  $\sim 5.0 \log_{10} \text{CFU/cm}^2$  with a maximum reduction of  $2.1 \log_{10} \text{CFU/cm}^2$  compared to the untreated control.

The alginate coating treatments consistently produced counts that were lower than the control throughout the entire storage (Fig. 4.1c). Plain alginate coating alone was able to slow down the growth of *Listeria monocytogenes*; with count  $2.5 \log_{10} \text{CFU/cm}^2$  lower than the control by the end of the storage. Except for 2.5% Opti.Form PD4, it appeared that incorporation of antimicrobials did not further enhance the antimicrobial effect exerted by the plain alginate coating. The coating treatment consisting of 2.5% Opti.Form PD4 was the most effective treatment, resulting in a  $3.5 \log_{10} \text{CFU/cm}^2$  reduction in *L. monocytogenes* counts that were significantly lower than those of the untreated control ( $P < 0.05$ ) at the end of the storage.

The inhibition effect of pectin coatings was similar to that of alginate coatings (Fig. 4.1d). Plain pectin coating alone was able to slow down growth of *Listeria monocytogenes* since counts were  $2.7 \log_{10} \text{CFU/cm}^2$  lower than those of the

control by the end of storage. Among the antimicrobial treatments, it appeared that 2.5% Opti.Form PD4/ 0.1% Protect M and 2.5% Optiform were the most effective, since counts of *Listeria monocytogenes* at the end of refrigeration storage were only slightly higher ( $0.3 \log \log_{10} \text{CFU/cm}^2$ ) than the initial inoculation levels ( $P < 0.05$ ). Since the pectin-based coatings demonstrated satisfactory inhibition effect in seven out of nine antimicrobial containing treatments, pectin was the carrier of choice for the second phase of the study.

It is worth noting that plain coatings (i.e starch, chitosan, alginate and pectin coatings in the absence of antimicrobials) were not always the least effective treatments. Protect-M, Guardian, and NovaGARD were often observed to be less effective compared to formulations involving salts of organic acids. The failure of Protect-M in controlling *Listeria monocytogenes* could be attributed to its physical property. The Protect-M liquid formulation reduced the viscosity of the coatings when it was mixed with the various coatings, preventing the coatings from effectively adhering to the samples upon surface-application. We also observed that while for the first 3 to 4 weeks, Guardian NR100 and NovaGARD exhibited satisfactory listeristatic activity, they progressively lost their inhibitory ability over time. Guardian and NovaGARD are both antimicrobial blends containing the active ingredient nisin. *Listeria monocytogenes* strain PSU1, included in our cocktail has been shown to demonstrate higher nisin resistance than other *Listeria monocytogenes* strains and it may exhibit resistance to nisin over time.

The treatments that recurrently appeared as two of the most effective were 2.5% OptiForm PD4 alone or in combination with 0.1% Protect M. OptiForm PD4 is a commercial formulation comprised of potassium lactate and SD. The antilisterial

ability of the lactate has been well documented (Devlieghere et al., 2001; Shelef, 1994). The main mechanisms of action of lactate salt reside in its ability to depress water activity (Debevere, 1989; Shelef, 1994) and to form lactic acid, a weak lipophilic acid that has the potential to cross the cell membrane in its molecular form and dissociate and acidify the cytoplasm once inside the cell interior (Salmond et al., 1984). In addition, the synergism existing between organic salts such as lactate and diacetate has previously been demonstrated (Schlyter et al., 1993; Mbandi and Shelef, 2002; Peirson et al., 2003; Juneja & Thippareddi, 2004; Neetoo et al., 2009). Salts of organic acids such as sodium lactate or potassium lactate and sodium diacetate are widely used by the processed poultry industry to control the growth *Listeria monocytogenes*, to extend the shelf-life and to enhance the flavor of products. Potassium and calcium lactate are equally effective as sodium lactate in controlling growth of bacteria including *Listeria* in meat products (Devlieghere et al., 2001); however, the use of potassium lactate over sodium lactate is more advantageous due to increased health problems associated with sodium.

**Effect of pectin coatings incorporating antimicrobials on the growth of *Listeria monocytogenes* on roasted turkey during frozen and subsequent refrigerated storage**

Table 4.3 shows the effect of pectin-based edible coatings in inhibiting growth of *Listeria monocytogenes* on roasted turkey during up to 4 weeks of frozen storage followed by 8 weeks of refrigerated storage. Virtually no change in *Listeria monocytogenes* populations was observed in the untreated control samples during frozen storage, indicating that *Listeria monocytogenes* was resistant to freezing. The coating treatments were able to slightly reduce the counts of *Listeria monocytogenes*

to values ranging from 2.4-3.0 log<sub>10</sub> CFU/cm<sup>2</sup>. During the subsequent 8-week refrigeration storage, *Listeria monocytogenes* in untreated controls grew to final populations of 4.3-5.7 log<sub>10</sub> CFU/cm<sup>2</sup>, which were lower than the 7.0 log population observed when frozen storage was not used (Table 4.1d). In addition, frozen storage before refrigeration enhanced the activity of all coating formulations, with counts almost all lower than the correspondent coating treatments when frozen storage was not used throughout the 8-week of storage. Overall, plain pectin coating was the least effective treatment. However, when combined with frozen storage, plain pectin coating was able to substantially slow down the growth of *Listeria monocytogenes*, with counts during the entire storage no more than 1.5 log<sub>10</sub> CFU/cm<sup>2</sup> higher than the initial inoculation level. Incorporation of antimicrobials further enhanced this inhibition effect in most of the cases. Overall, treatments including 2.5% OptiForm were the most effective; with the final *Listeria monocytogenes* concentration ranging from 2.0 log<sub>10</sub> CFU/cm<sup>2</sup> to 3.6 log<sub>10</sub> CFU/cm<sup>2</sup> by the end of the 8-week refrigerated storage. Treatments, ranked in the following order of decreasing efficacy: 2.5% OptiForm > 0.25% SD > 2.5% OptiForm/ 0.2% Protect M > 2% SL/ 0.25% SD > 0.25% NovaGARD > 2% SL (P<0.05).

Except for the coating treatment containing SD at week 8 of refrigeration storage, the length of frozen storage did not significantly affect the counts of *Listeria monocytogenes* for the other coating treatments during the entire refrigeration storage. Palumbo and Williams (1991) previously reported a delay in growth of *Listeria monocytogenes* in thawed and chilled products following frozen storage (12 days at -22°C). *Listeria monocytogenes* was able to grow in both treated and untreated samples because this bacterium is psychrotrophic (Bell, et al., 1995). However, the

concerted application of freezing and antimicrobials significantly reduced the extent of population growth at refrigeration temperature. This demonstrates that frozen storage in conjunction with antimicrobial coatings can represent effective hurdles in food safety.

**Aerobic and anaerobic study on roasted turkey coated with pectin coating solutions incorporating GRAS antimicrobials during freezing - refrigerated storage**

The effect of the treatments on the total aerobic and aerobic population is presented in Table 4.4. The total aerobic counts increased from 2.4 log<sub>10</sub> CFU/cm<sup>2</sup> to an average of 5.0 log<sub>10</sub> CFU/cm<sup>2</sup>, 4.7 log<sub>10</sub> CFU/cm<sup>2</sup>, 5.6 log<sub>10</sub> CFU/cm<sup>2</sup> and 5.0 log<sub>10</sub> CFU/cm<sup>2</sup> after 1, 2, 3 and 4 weeks of frozen storage plus 8 weeks of refrigerated, respectively. The initial anaerobic counts were 0.70 log<sub>10</sub> CFU/cm<sup>2</sup>, and increased to 5.4, 5.3, 5.6 and 5.1 log<sub>10</sub> CFU/cm<sup>2</sup> after 1, 2, 3 and 4 weeks of frozen plus 8 weeks of refrigerated storage, respectively. Significant differences were observed between most of the treatments and the untreated control, but no statistical differences were recognized within similar treatment groups with different frozen storage duration (P > 0.05). Similarly, for all treatments under the different frozen storage times, there was no significant difference between the treated and untreated samples in their anaerobic counts except for samples treated with 0.25% NovaGARD at 2 weeks of frozen followed by refrigeration. According to Mejlholm et al's (2005), gram-positive, acid tolerant lactic acid bacteria (LAB) tend to predominate as the main spoilage microflora. The main LAB strains associated with spoilage of cooked meat products belong to the genera of *Lactobacillus* and *Leuconostoc* (Holzapfel, 1998; Samelis et al., 2000). Mejlholm et al's (2005) reported that frozen storage had no minimal effect

on their maximum specific growth rates and maximum cell densities. Since LAB are facultatively anaerobic, they may have resulted in a similar growth profile for both aerobic and anaerobic count determinations. Hence, the application of antimicrobial edible coating in combination with a frozen storage hurdle had limited inhibitory effect against background microflora prevalent on roasted turkey.

### **CONCLUSION**

This study demonstrates that pectin-based coatings incorporating various antimicrobials hold great promise when used as a post-process surface-treatment to enhance the safety of ready to eat turkey. In addition, this study highlighted the effectiveness of pectin-based edible coatings incorporating various novel combinations of antimicrobials, against the growth of *Listeria monocytogenes* on roasted turkey during refrigerated storage. Finally, the study clearly demonstrated the efficacy of a concerted application of antimicrobial treatments, frozen and refrigerated storage in achieving an appreciable inhibition of *Listeria monocytogenes*.

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**FIGURE**

Fig. 4.1 Effect of edible coatings incorporating GRAS antimicrobials on the growth of *Listeria monocytogenes* on vacuum packaged roasted turkey stored at 4°C. (a) starch, (b) chitosan, (c) alginate and (d) pectin.

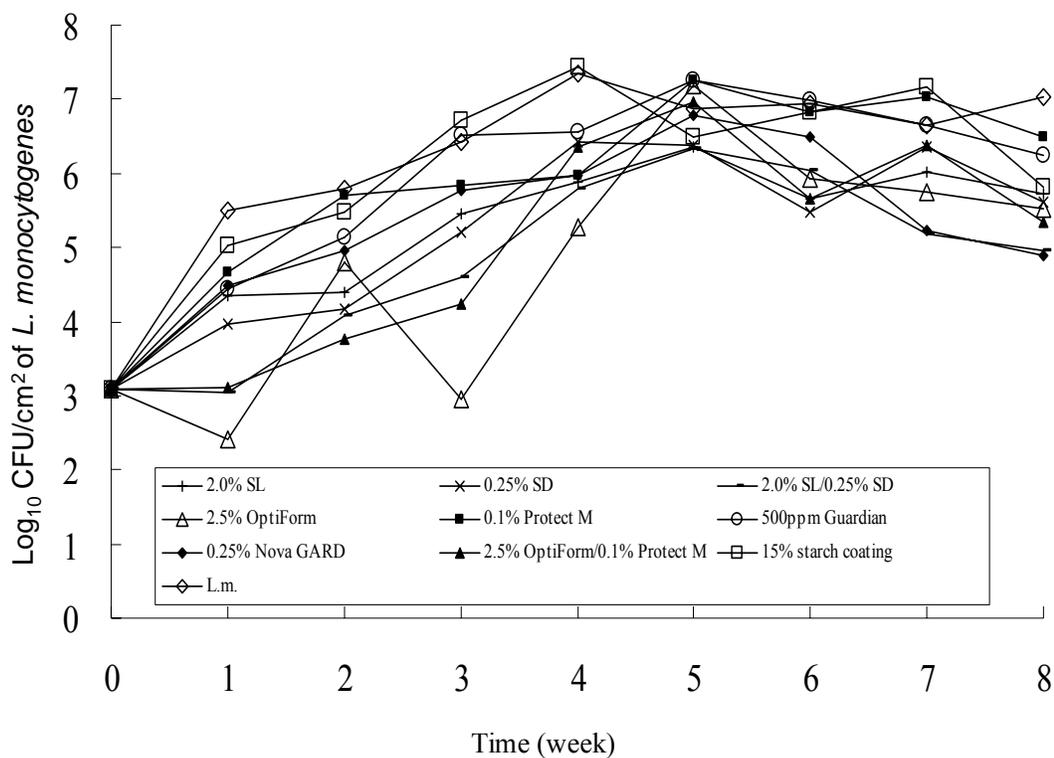


Fig. 4.1a

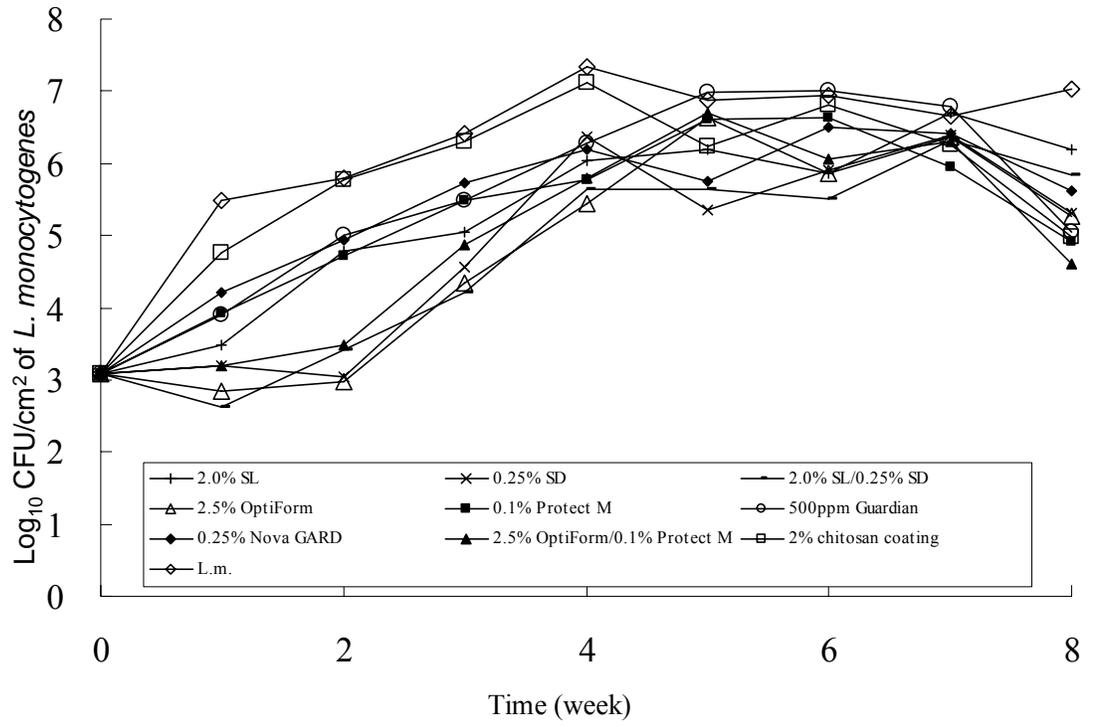


Fig. 4.1b

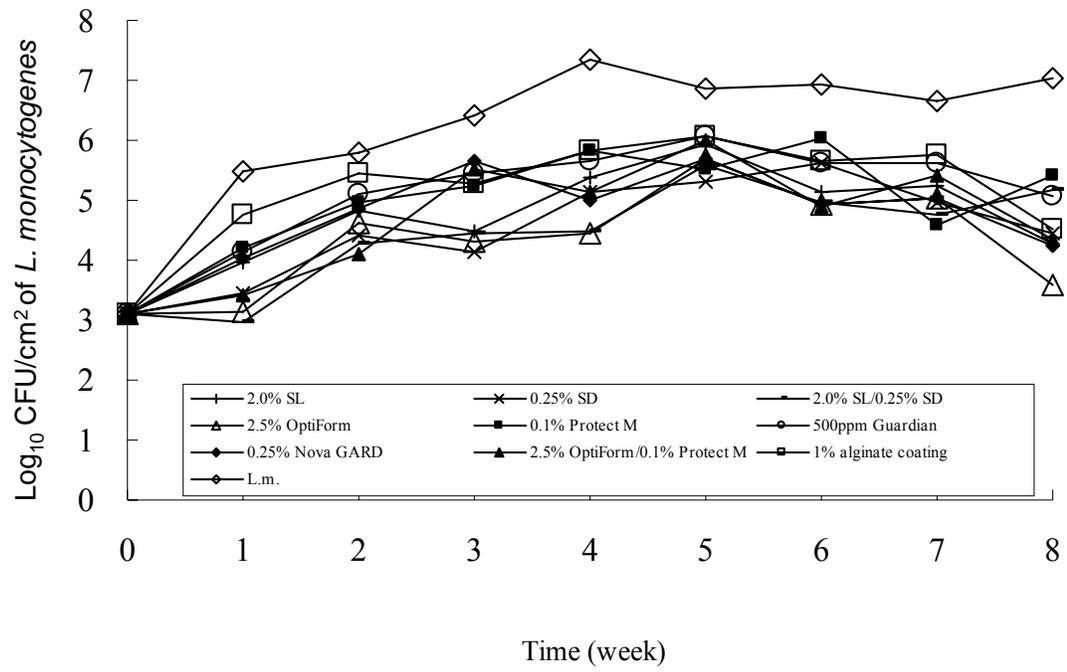


Fig. 4.1c

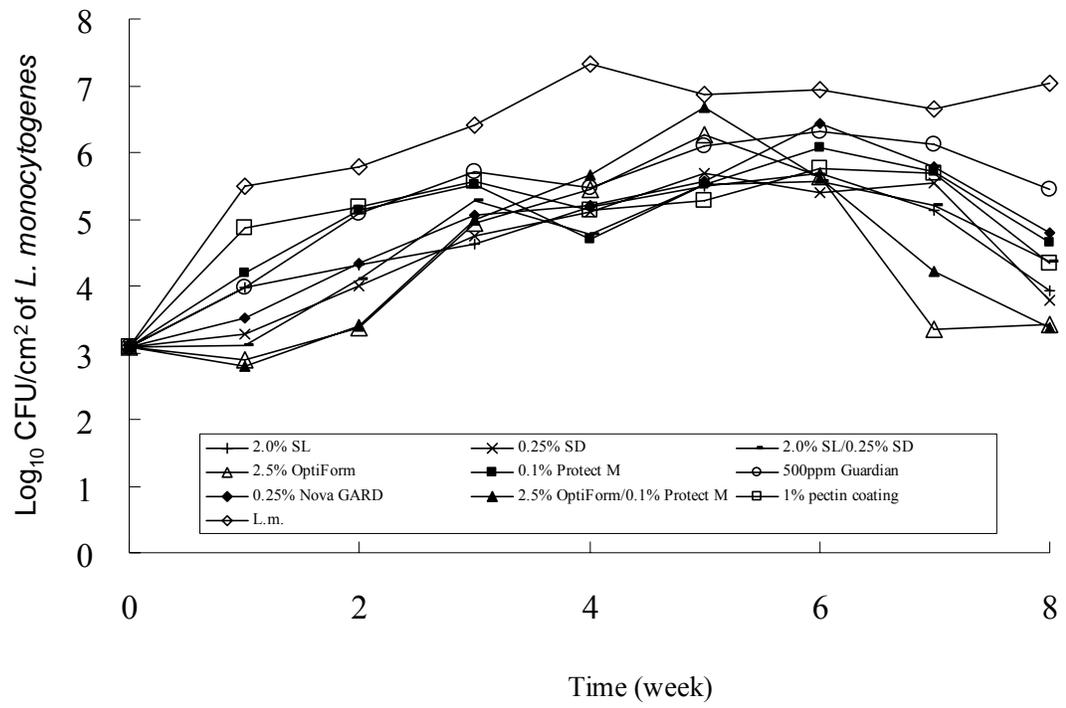


Fig. 4.1d

**TABLE**

Table 4.1 Antimicrobial concentrations (%weight of antimicrobials/weight of roasted turkey) in varied coatings applied onto roasted turkey.

Sample Designation	SL (%)	SD (%)	OptiForm (%)	Protect-M (%)	Guardian NR100 (ppm)	Nova GARD (%)
1	2	0	0	0	0	0
2	0	0.25	0	0	0	0
3	2	0.25	0	0	0	0
4	0	0	2.5	0	0	0
5	0	0	0	0.1	0	0
6	0	0	0	0	500	0
7	0	0	0	0	0	0.25
8	0	0	2.5	0.1	0	0
9	0	0	0	0	0	0
10				No coating		

The SL concentrations refer to pure SL.

Table 4.2 Antimicrobial concentrations (%weight of antimicrobials/weight of roasted turkey) in pectin coatings applied onto roasted turkey.

Sample Designation	SL (%)	SD (%)	OptiForm (%)	Protect-M (%)	Nova GARD (%)
1	2	0	0	0	0
2	0	0.25	0	0	0
3	2	0.25	0	0	0
4	0	0	2.5	0	0
5	0	0	0	0	0.25
6	0	0	2.5	0.2	0
7	0	0	0	0	0
8	No coating				

The SL concentrations refer to pure SL.

Table 4.3 Effect of pectin-based edible coatings incorporating GRAS antimicrobials on the growth of *Listeria monocytogenes* on vacuum packaged roasted turkey stored at up to 4 weeks of frozen storage followed by up to 8 weeks of refrigerated storage. The initial *Listeria monocytogenes* level was 3.1 log<sub>10</sub> CFU/cm<sup>2</sup>. Data are the means of three replicates ± one standard deviation (log<sub>10</sub> CFU/cm<sup>2</sup>).

Treatment	Duration of Frozen Storage			
	1 week	2 week	3 week	4 week
<u>0 week refrigeration</u>				
control	3.0±0.1 <sup>aA</sup>	3.0±0.1 <sup>aA</sup>	3.1±0.2 <sup>aA</sup>	2.9±0.1 <sup>aA</sup>
1% pectin coating	3.0±0.1 <sup>aA</sup>	2.9±0.1 <sup>abA</sup>	3.0±0.2 <sup>abA</sup>	2.9±0.1 <sup>aA</sup>
2.0%SL	2.7±0.1 <sup>abA</sup>	2.7±0.3 <sup>abA</sup>	2.8±0.2 <sup>abA</sup>	2.7±0.2 <sup>aA</sup>
0.25%SD	2.9±0.1 <sup>aA</sup>	2.8±0.2 <sup>abA</sup>	2.8±0.2 <sup>abA</sup>	2.8±0.1 <sup>aA</sup>
2.0% SL/0.25% SD	2.9±0.04 <sup>aA</sup>	2.9±0.1 <sup>abA</sup>	2.8±0.1 <sup>abA</sup>	2.9±0.1 <sup>aA</sup>
2.5%OptiForm	3.0±0.1 <sup>aA</sup>	2.9±0.1 <sup>aA</sup>	2.9±0.2 <sup>abA</sup>	2.9±0.1 <sup>aA</sup>
0.25%NovaGARD	2.5±0.2 <sup>bA</sup>	2.4±0.2 <sup>bA</sup>	2.5±0.2 <sup>bA</sup>	2.4±0.05 <sup>bA</sup>
2.5% Optiform + 0.2% ProtectM	2.8±0.1 <sup>aA</sup>	2.8±0.1 <sup>abA</sup>	2.8±0.04 <sup>abA</sup>	2.9±0.1 <sup>aA</sup>
<u>2 week refrigeration</u>				
control	5.0±0.4 <sup>aA</sup>	4.9±1.1 <sup>aA</sup>	5.5±0.3 <sup>aA</sup>	5.1±0.5 <sup>aA</sup>
1% pectin coating	3.5±0.6 <sup>bA</sup>	3.8±0.7 <sup>aA</sup>	3.6±0.3 <sup>bcA</sup>	3.6±0.8 <sup>abA</sup>
2.0%SL	3.1±0.3 <sup>bA</sup>	3.7±1.1 <sup>aA</sup>	4.1±0.1 <sup>bA</sup>	3.4±0.9 <sup>abA</sup>
0.25%SD	2.8±0.2 <sup>bA</sup>	3.9±1.0 <sup>aA</sup>	3.5±0.5 <sup>bcA</sup>	3.6±0.7 <sup>abA</sup>
2.0% SL/0.25% SD	2.7±0.1 <sup>bA</sup>	3.6±1.3 <sup>aA</sup>	3.0±0.3 <sup>cA</sup>	3.4±0.5 <sup>abA</sup>
2.5%OptiForm	2.8±0.4 <sup>bA</sup>	3.7±1.2 <sup>aA</sup>	3.4±0.76 <sup>bcA</sup>	3.5±0.4 <sup>abA</sup>

0.25%NovaGARD	3.4±0.3 <sup>ba</sup>	3.9±1.3 <sup>aA</sup>	3.3±0.3 <sup>bcA</sup>	3.2±0.8 <sup>ba</sup>
2.5% Optiform + 0.2% ProtectM	2.7±0.3 <sup>ba</sup>	3.0±0.4 <sup>aA</sup>	2.9±0.1 <sup>ca</sup>	3.1±0.2 <sup>ba</sup>
<u>4 week refrigeration</u>				
control	5.5±0.8 <sup>aA</sup>	5.7±0.5 <sup>aA</sup>	5.7±0.2 <sup>aA</sup>	5.2±0.6 <sup>aA</sup>
1% pectin coating	4.5±0.4 <sup>abA</sup>	3.8±0.39 <sup>ba</sup>	4.2±0.5 <sup>abA</sup>	3.6±0.3 <sup>abA</sup>
2.0%SL	4.1±0.9 <sup>abA</sup>	4.4±0.7 <sup>abA</sup>	4.1±0.3 <sup>abA</sup>	4.5±0.2 <sup>abA</sup>
0.25%SD	4.6±0.2 <sup>abA</sup>	3.7±0.6 <sup>ba</sup>	3.8±1.0 <sup>ba</sup>	3.9±0.2 <sup>abA</sup>
2.0% SL/0.25% SD	3.6±0.1 <sup>ba</sup>	3.3±0.5 <sup>ba</sup>	3.1±0.4 <sup>ba</sup>	3.1±0.9 <sup>ba</sup>
2.5%OptiForm	4.5±0.4 <sup>abA</sup>	3.6±0.4 <sup>ba</sup>	3.5±1.0 <sup>ba</sup>	3.9±0.7 <sup>abA</sup>
0.25%NovaGARD	4.2±0.3 <sup>abA</sup>	3.4±0.1 <sup>ba</sup>	3.5±0.8 <sup>ba</sup>	3.8±0.7 <sup>abA</sup>
2.5% Optiform + 0.2% ProtectM	3.9±0.8 <sup>ba</sup>	3.4±0.6 <sup>ba</sup>	3.3±0.3 <sup>ba</sup>	3.6±0.7 <sup>abA</sup>
<u>6 week refrigeration</u>				
control	5.3±1.0 <sup>aA</sup>	4.9±0.5 <sup>aA</sup>	5.2±0.6 <sup>aA</sup>	5.6±1.1 <sup>aA</sup>
1% pectin coating	4.2±1.0 <sup>aA</sup>	3.0±0.1 <sup>abA</sup>	4.1±0.3 <sup>abA</sup>	4.1±0.5 <sup>abA</sup>
2.0%SL	4.0±1.2 <sup>aA</sup>	3.5±0.8 <sup>abA</sup>	3.9±0.4 <sup>abA</sup>	4.3±1.2 <sup>abA</sup>
0.25%SD	4.3±0.9 <sup>aA</sup>	2.6±0.3 <sup>ba</sup>	3.6±1.0 <sup>abA</sup>	3.1±1.3 <sup>ba</sup>
2.0% SL/0.25% SD	4.2±1.1 <sup>aA</sup>	3.4±1.2 <sup>abA</sup>	3.8±1.0 <sup>abA</sup>	2.7±0.3 <sup>ba</sup>
2.5%OptiForm	3.1±1.0 <sup>aA</sup>	2.4±0.7 <sup>ba</sup>	3.5±0.8 <sup>abA</sup>	3.8±0.7 <sup>abA</sup>
0.25%NovaGARD	3.8±1.8 <sup>aA</sup>	2.6±0.9 <sup>ba</sup>	4.1±0.4 <sup>abA</sup>	3.2±0.4 <sup>ba</sup>
2.5% Optiform + 0.2% ProtectM	3.6±1.3 <sup>aA</sup>	2.6±0.8 <sup>ba</sup>	3.0±0.9 <sup>ba</sup>	2.4±0.6 <sup>ba</sup>
<u>8 week refrigeration</u>				
control	5.7±0.2 <sup>aA</sup>	5.5±0.2 <sup>aAB</sup>	4.3±0.5 <sup>aB</sup>	4.8±0.9 <sup>aAB</sup>
1% pectin coating	4.1±1.2 <sup>aA</sup>	3.5±0.1 <sup>ba</sup>	3.2±0.4 <sup>abA</sup>	3.1±0.7 <sup>ba</sup>
2.0%SL	3.8±0.1 <sup>aA</sup>	3.6±1.2 <sup>ba</sup>	2.6±0.3 <sup>ba</sup>	2.4±0.2 <sup>ba</sup>

0.25%SD	3.8±1.3 <sup>aA</sup>	2.9±0.2 <sup>bAB</sup>	2.3±0.5 <sup>bAB</sup>	2.0±0.3 <sup>bB</sup>
2.0% SL/0.25% SD	3.9±1.0 <sup>aA</sup>	2.4±0.6 <sup>bA</sup>	2.8±0.2 <sup>bA</sup>	2.3±0.5 <sup>bA</sup>
2.5%OptiForm	3.2±0.9 <sup>aA</sup>	3.6±0.7 <sup>bA</sup>	2.4±0.6 <sup>bA</sup>	2.0±0.4 <sup>bA</sup>
0.25%NovaGARD	3.8±1.3 <sup>aA</sup>	3.6±0.1 <sup>bA</sup>	3.3±0.6 <sup>abA</sup>	2.3±0.5 <sup>bA</sup>
2.5% Optiform + 0.2% ProtectM	3.4±1.5 <sup>aA</sup>	2.8±0.3 <sup>bA</sup>	2.7±0.1 <sup>bA</sup>	2.2±0.4 <sup>bA</sup>

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Table 4.4 Effect of pectin-based edible coatings incorporating GRAS antimicrobials on the growth of aerobic (4.4a) and anaerobic (4.4b) microflora on vacuum packaged roasted turkey stored at up to 4 weeks of frozen storage followed by 8 weeks of refrigerated storage. The initial aerobic and anaerobic counts were 2.3 and 0.7 log<sub>10</sub> CFU/cm<sup>2</sup>, respectively. Data are the means of three replicates ± one standard deviation (log<sub>10</sub> CFU/cm<sup>2</sup>) at the end of 8-week refrigerated storage.

4.4a

Treatment	Duration of Frozen Storage			
	1 week	2 week	3 week	4 week
no coating control	6.1±0.3 <sup>aA</sup>	6.0±0.5 <sup>aA</sup>	6.1±0.4 <sup>aA</sup>	6.3±0.1 <sup>aA</sup>
1% pectin coating	5.2±0.6 <sup>aA</sup>	5.2±0.2 <sup>aA</sup>	5.7±0.1 <sup>aA</sup>	5.7±0.1 <sup>abA</sup>
2.0%SL	4.5±0.6 <sup>aA</sup>	5.2±0.4 <sup>aA</sup>	5.4±0.6 <sup>aA</sup>	4.7±0.5 <sup>bA</sup>
0.25%SD	4.7±0.4 <sup>AB</sup>	5.0±0.2 <sup>aAB</sup>	5.7±0.1 <sup>aA</sup>	5.0±0.5 <sup>bAB</sup>
2.0% SL/0.25% SD	5.1±1.1 <sup>aA</sup>	5.0±0.8 <sup>aA</sup>	5.7±0.8 <sup>aA</sup>	4.9±0.7 <sup>bA</sup>
2.5%OptiForm	5.0±1.0 <sup>aA</sup>	4.7±1.0 <sup>aA</sup>	5.0±0.7 <sup>aA</sup>	4.7±0.4 <sup>bA</sup>
0.25%NovaGARD	5.2±0.9 <sup>aA</sup>	4.0±1.1 <sup>aA</sup>	5.7±0.3 <sup>aA</sup>	4.8±0.1 <sup>bA</sup>
2.5% Optiform + 0.2% ProtectM	5.0±0.2 <sup>aA</sup>	4.1±2.1 <sup>aA</sup>	6.0±0.4 <sup>aA</sup>	5.2±0.4 <sup>abA</sup>

## 4.4b

Treatment	Duration of Frozen Storage			
	1 week	2 week	3 week	4 week
no coating control	6.1±0.3 <sup>aA</sup>	5.9±0.2 <sup>aA</sup>	6.1±0.1 <sup>aA</sup>	6.1±0.04 <sup>aA</sup>
1% pectin coating	5.7±0.4 <sup>abA</sup>	5.7±0.3 <sup>abA</sup>	5.8±0.2 <sup>abA</sup>	5.7±0.2 <sup>abA</sup>
2.0%SL	5.3±0.3 <sup>abA</sup>	5.6±0.2 <sup>abA</sup>	5.4±0.3 <sup>bA</sup>	5.0±0.4 <sup>bcA</sup>
0.25%SD	5.0±0.3 <sup>bA</sup>	5.01±0.6 <sup>abA</sup>	5.5±0.3 <sup>abA</sup>	5.3±0.2 <sup>abcA</sup>
2.0% SL/0.25% SD	5.1±0.7 <sup>abA</sup>	5.5±0.4 <sup>abA</sup>	5.6±0.3 <sup>abA</sup>	4.7±0.6 <sup>cA</sup>
2.5%OptiForm	5.8±0.3 <sup>abA</sup>	5.7±0.4 <sup>abA</sup>	5.6±0.3 <sup>abA</sup>	5.1±0.3 <sup>abcA</sup>
0.25%NovaGARD	5.2±0.3 <sup>abAB</sup>	4.4±0.9 <sup>bB</sup>	5.8±0.2 <sup>abA</sup>	4.9±0.1 <sup>bcAB</sup>
2.5% Optiform + 0.2% ProtectM	5.4±0.1 <sup>abA</sup>	5.5±0.6 <sup>abA</sup>	5.9±0.1 <sup>abA</sup>	5.2±0.7 <sup>abcA</sup>

Values in the same column followed by the same lower-case letter are not significantly different ( $P > 0.05$ ).

On each sampling day, values in the same row followed by the same upper-case letter are not significantly different ( $P > 0.05$ ).

## **Chapter 5**

### **FUTURE RESEARCH**

In general, multiplication of *Listeria monocytogenes* on fishery products such as cold-smoked salmon and meat products such as roasted turkey could be inhibited by surface treatments such as the application of edible antimicrobial films and coatings. A synergistic hurdle would include pre-frozen storage to further inhibit *Listeria monocytogenes* in processed food.

Further determination of the optimum length of frozen storage on different kinds of food products is valuable. Moreover, customers are always looking for less processed food. Use of lower levels of antimicrobials in food products is desirable. Thus, in order to reach ideal efficacy using minimal antimicrobials, we need to develop research in adjusting the composition, formula and the dose in antimicrobials.

Since the hurdles adopted in the research cannot be applied to all types of RTE foods including some cold sensitive products. To better satisfy the needs of the consumers as well as the producers and reduce energy cost (Ashie et al., 1996), alternative methods are worthwhile investigated and applied. Modified atmosphere packaging (MAP) is another representative of antimicrobial packaging, which has become a useful preservation technique for prolonging the shelf time of perishable products and an effective inhibition to bacteria. MAP uses oxygen, nitrogen and carbon dioxide as the most usual gases (Farber, 1991; Church and Parsons, 1995; Gimenez et al., 2002). Addition of carbon dioxide and removal of oxygen reduce catabolic activity and microbial growth. In general, Carbon dioxide could retard

microbial growth of gram-positive bacteria, though the bacteria are resistant to it (Hendricks and Hotchkiss, 1997). Studies have demonstrated that a modified atmosphere consisting of 100% CO<sub>2</sub> is inhibitory to the growth of *Listeria monocytogenes* (Gill and Reichel, 1989; Razavilar and Genigeorgis, 1992; Lyver et al., 1998). Farber and Daley (1994) reported that 70% CO<sub>2</sub> also caused a reduction in *Listeria monocytogenes* growth rate in packaged turkey roll slices stored at both 4 and 10°C. But considering the fact that CO<sub>2</sub> dissolved into the meat juice lead to deformation of the package and discoloration of meat products, establishment of gas mixtures need to be modified and attempts have been made to replace part of the CO<sub>2</sub> with O<sub>2</sub> and or N<sub>2</sub> (Stenström, 1985). The use of MAP is likely to be another application of hurdle in *Listeria monocytogenes* inhibition, and that in the future the range of foods using MAP system will expand. Further studies on the potential antilisterial activity of MAP combined with antimicrobial edible coating/film and frozen storage should be developed.

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