EDIBLE COATINGS TO CONTROL THE GROWTH OF LISTERIA MONOCYTOGENES ON POACHED AND DELI TURKEY PRODUCTS AND THE EFFECT OF GROWTH CONDITIONS ON THE PRESSURE RESISTANCE, INACTIVATION, AND RECOVERY OF PATHOGENS

by

Gregory Juck

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by
Gregory Juck

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: _____
Charles G. Riordan, Ph.D.
Vice Provost for Graduate and Professional Education

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ABSTRACT

The relatively high prevalence of *Listeria monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium in various ready-to-eat (RTE) food products is of great concern to the food industry. The overall objective of this project was to assess the efficacy of edible coating and high hydrostatic pressure (HHP) processing technologies to enhance the safety of RTE turkey products. In the first phase of the study, we compared the antimicrobial efficacy of polysaccharide-based edible coatings (alginate, carrageenan, pectin, xanthan gum, and starch) incorporating nisin (500 IU/g), sodium lactate (SL, 2.4%), sodium diacetate (SD, 0.25%), and potassium sorbate (PS, 0.3%) and the commercial products, Novagard™ CB1 (0.25%) and Guardian™ NR100 (500 ppm) to inhibit *L. monocytogenes* on deli turkey. The coatings were applied onto the surface of deli turkey discs inoculated with ~ 3 log CFU/g of *L. monocytogenes* and stored at 4°C for 30 days. The most effective treatments were alginate-based coatings supplemented with SL (2.4%)/PS (0.3%) which delayed growth of the pathogen with final counts reaching 1.2-5.6 log CFU/g lower than the control untreated samples. In the second part of the project, the influence of growth and recovery temperatures (15, 25, 35, and 40°C), pressure levels (400 and 600 MPa), treatment temperatures (4, 20, and 40°C) and recovery gaseous conditions (aerobic and anaerobic) on pressure

resistance of *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* was investigated. Pressure inactivation of the bacterial pathogens was shown to increase as a function of the pressure levels and treatment temperatures. The temperature history and physiological age of the bacterial culture also influenced their pressure resistances. In general, early stage cells (representative of exponential phase) were more baro-resistant than late stage cells (representative of stationary phase) ($P < 0.05$). Aerobic and anaerobic recovery conditions were not found to have any appreciable effect on bacterial recovery ($P > 0.05$). In addition, recovery temperatures ≤ 35°C promoted greater recovery of injured stationary phase cells than higher temperatures ($P < 0.05$). Overall, HHP treatments at the 40°C recovery temperature, 15°C growth temperature, 600 MPa, 40°C treatment temperature, and stationary phase were the most promising, delivering 5.3 to 7.7 log CFU/g reduction of *Salmonella, E. coli*, and *L. monocytogenes*. This study thus highlights the effectiveness of two intervention technologies that can be applied to foods such as RTE turkey that are susceptible to post-processing recontamination.

Chapter 1

INTRODUCTION

Listeria monocytogenes, Escherichia coli O157:H7 *and Salmonella enteric* serovar Typhimurium are all serious safety concerns for the food industry, especially with respect to ready-to-eat (RTE) and fresh meat products. *L. monocytogenes*, a gram-positive, non-spore-forming rod-shaped bacterium, is responsible for the food borne illness listeriosis. *Escherichia coli* O157:H7 is a gram-negative rod-shaped bacterium. *Salmonella* Typhimurium is a gram-negative rode-shaped bacterium. *Salmonella* Typhimurium can often cause the foodborne illness salmonellosis. The three pathogens are responsible for a large number of the food-borne illnesses today. Combined it is estimated that nontyphoidal *Salmonella*, *L. monocytogenes*, and *E. coli* O157: H7 cause approximately 1.65 million annual domestically acquired illnesses in the United States each year (CDC, 2000; Scallen et., al; 2011).

A variety of food products have been implicated in outbreaks of *L. monocytogenes, S.* Typhimurium, and *E. coli* including meats, produce, and dairy. The ubiquitous nature of *L. monocytogenes* can be attributed to its growth characteristics. *L. monocytogenes* can tolerate a high salt level of up to 16%; it is psychrotrophic with a minimum growth temperature of -0.4°C; it grows in a wide pH range of 4.2-9.6 and is facultatively anaerobic (Farber, 1991). *S.* Typhimurium has a minimum growth pH of approximately 4 (Chung and Goepfert, 2006) and a minimum growth temperature of approximately 6°C (Matches and Liston, 2006). Also the bacteria can tolerate a salt content of 8% (Thayer et al., 1987). *E. coli* has a minimum growth pH

of approximately 4 (Presser et al., 1997), a minimum growth temperature of approximately 7.5°C (Shaw et al., 1971), and can tolerate a salt content of 6.5% (Sutherland et al., 1995). The growth requirements of each bacterium are easily met by numerous food groups such as RTE poultry products including cooked turkey which is characterized by a salt content of < 2%, a pH of about 6.5, a storage temperature of around 4°C and a vacuum-packaging environment. In addition, *L. monocytogenes* is known to be able to re-contaminate cooked meat or poultry products during the processing and packaging steps (Janes et al., 2002).

RTE meat and poultry products are fully processed and should be free of pathogens (Huang, 2004). However, various instances of foodborne disease outbreaks have been reported in recent years, linked with pathogens, notably *L. monocytogenes* (CDC 1998; CDC 2000; CDC 2002). Although thermal processes are used in the food industry with the goal to eliminate *L. monocytogenes* from RTE meat and poultry products, cross-contamination can occur. During a joint study conducted at a turkey frankfurter production site, researchers from the U.S. Centers for Disease Control and Prevention and the U.S. Department of Agriculture identified that the operation of peeling immediately following thermal processing was the main critical stage where recontamination of franks by *L. monocytogenes* took place before final packaging (Wenger et al., 1990). Hence, RTE poultry products contaminated with this potentially deadly pathogen could enter the market, causing food poisoning especially among the high risk groups of the population. Contamination by *Salmonella and E. coli* O157:H7 of foods processed by low temperature cooking have also been reported (Huang, 2004).

One possible solution to this contamination problem is the use of antimicrobials. The antimicrobials can be incorporated into the food matrix in several ways; the antimicrobials could be incorporated directly into the food formulation, the food could be dipped into an antimicrobial

solution, or the food surface could be covered with an antimicrobial coating. The inclusion of antimicrobials into meat formulations to inhibit the growth of *L. monocytogenes* has previously been reported (Barmpalia et al., 2004; 2005).

Also high pressure processing can be effectively used to pasteurize raw or cooked meat products in their final packaging while preserving their organoleptic and nutritional qualities. Since the food and the package are treated together, post-processing contamination can be prevented. Several researchers have investigated the efficacy of HHP on inactivating foodborne pathogens *L. monocytogenes* and *Salmonella* on vacuum-packaged RTE meat and poultry products to enhance their microbiological safety (Garriga and Aymerich, 2009). Similarly, the commercial use of Safe Pac™ high pressure pasteurization to inactivate microorganisms and extend the shelf-life of pre-packaged RTE products, including meats, soups, wet salads, sauces, fruit smoothies, shellfish and seafood, by 200-300% has been reported (Safe Pac LLC, 2010). Jofré et al. (2008a; b) and Marcos et al. (2008) demonstrated the synergistic effect of HHP and antimicrobial packaging incorporating natural antimicrobials to control *Salmonella* on sliced cooked ham. Jofré et al. (2008b) also demonstrated that antimicrobial packaging, HHP and refrigerated storage acted as an effective triple combination of hurdles to inhibit *Salmonella* sp. and *L. monocytogenes* in cooked ham. Another innovative example of "hurdle technology" involves the use of HHP at 300 to 800 MPa to treat foods in contact with an antimicrobial packaging material to ensure a delayed release of the antibacterial compound, allyl isothiocyanate (AIT). AIT was encapsulated within cyclodextrins inside a polylactic acid (PLA) matrix to ensure a controlled release of the agent during ambient temperature storage (INRA, 2010). However, determination of adequate pressure processing parameters and characterization of

bacterial growth parameters such as culture and recovery temperatures and gas atmospheres for maximal recovery are need to ensure that optimum processing conditions are selected.

The objectives of this study were to: (i) to develop effective antimicrobial edible coatings to control the growth of *L. monocytogenes* on several turkey products formulated or processed differently, (ii) determine the effect of prior growth temperature and growth phase on the pressure resistance of *L. monocytogenes*, *Salmonella* Typhimurium and *E. coli* O157:H7 prior to pressure treatment, (iii) determine the recovery or incubation temperature and gas atmosphere after pressure treatment on the recovery of the pathogens, and (iv) determine the effect of pressure treatment temperature on the inactivation of the pathogens.

Chapter 2

LITERATURE REVIEW

Listeria monocytogenes

History

The history of *Listeria monocytogenes* begins in 1911 when a Swedish microbiologist, Hulphers, isolated a bacterium causing necrosis of the liver of a rabbit. Hulphers named the bacterium *Bacillus* hepatitis because of its relation to the liver (Huplhers 1911). Then in 1926 Murray and his colleagues found the same bacterium in several rabbits and guinea pigs. Murray and his colleagues named the bacterium *Bacillus monocytogenes* because of the bacterium's ability to cause a large amount of monocytosic activity (Murray et al., 1926). The following year Pirie had found the same microbacteria in gerbils; the bacterium was named *Tiger river bacillus* since it was first found near the Tiger River in South Africa. Later, Pirie had realized that Murray and his colleagues found the same bacterium earlier; Pirie then changed the name of the organism to *Listerella monocytogenes*, in honor of British surgeon, Lord Joseph Lister. In 1940, it was noticed that the name Listerella had been used previously for a group of molds and Pirie changed the name to *L. monocytogenes* (Pirie 1940). The latest name then went into the 6th edition of *Bergey's Manual of Determinative Bacteriology* (Bergey and Breed 1948). The first incidence of *L. monocytogenes* being reported in humans was in 1929 when Nyfeldt was the first to isolate the bacterium from a person. In addition to this first species in the *Listeria* genus five other species exist including, *L. innocua, L. welshimeri, L. seelegeri, L. grayi, and L. ivanovii.*

(Boerlin et al., 1992). In the 1980's an increase in the number of illnesses and outbreaks caused by *L. monocytogenes* established the pathogen as a serious foodborne concern (McLauchlin 1996a; b). Today the pathogen is still a serious concern and requires further research to adequately reduce the instance of *L. monocytogenes* outbreaks and illnesses.

Morphology

L. monocytogenes is a gram-positive non-spore-forming rod-shaped bacterium. The bacterium is also facultatively aerobic, catalase-positive, oxidase-negative, and acapsular. The rods of the bacterium measure 1.0 to 2.0 µm in length and 0.4 to 0.5 µm in diameter. Also the bacterium is motile by polar peitrichous flagella and exhibits a tumbling motility especially at the 20 to 25^{o}C range (Gray and Killinger 1966). When cultured on nutrient agar (after 24 hours of incubation) *L. monocytogenes* colonies are round, 0.5 to 1.5 mm in diameter, translucent, and have a smooth glistening surface (S-form). During prolonged incubation (for 3 to 7 days) may appear 3 to 5 mm in size and in the rough form (R-form). Also *L. monocytogenes* exhibits a narrow zone of β-haemolysis around colonies when grown on blood agar. When the bacterium is viewed with oblique lighting the colonies usually appear blue or green, but appears orange or yellow when grown on blood free agar.

Growth Requirements

Growth of *L. monocytogenes* occurs from 0.5^{o}C (Juntilla et al., 1988) to 45^{o}C (Petron and Zottola, 1989), but the optimum growth temperature for the bacterium is 30 to 37^{o}C. It has been demonstrated that the bacterium can grow at -2^{o}C in laboratory medium broth (Bajard et al., 1996). Temperature fluctuation can enhance the thermo-tolerance of *L. monocytogenes* (Farber and Peterkin 1991). The bacterium can grow in salt levels as high as 10% sodium chloride (Seelinger, 1961). *L. monocytogenes* grows best at slightly alkaline to neutral conditions but

grows readily from pH 4.6 to 9.6 with an optimum of approximately 7.1 (Seelinger, 1961; Gray and Killinger, 1966; AFSSA, 2000).

For the bacterium to grow several nutrients are required including, riboflavin, biotin, thiamine, thioctic acid, and some amino acids (Pearson and Marth 1990). *L. monocytogenes* requires carbohydrate as the primary energy source for growth with glucose being the preferred source (Pine et al., 1989; Premaratne et al., 1991). The end products of the bacterium metabolism vary based on conditions with lactate and acetate being produced under both aerobic and anaerobic conditions, but acetoin is produced only under aerobic conditions (Romick et al., 1996). Also *L. monocytogenes* can grow in the presence of CO_2 at low temperatures; however, higher concentrations of the gas (above 70% CO_2) can inhibit the bacterium at temperatures below 7^oC. It has been shown though that even with high CO_2 concentrations (70%) and in the presence of just 5% O_2, the bacterium is able to grow (Wimpfheimer et al., 1990).

Human Listeriosis

L. monocytogenes can cause general fever and flu-like symptoms for most people, including nausea, vomiting, and diarrhea (CFSAN, 2000). Also the bacterium can lead to more severe complications such as meningitis and septicemia (Salamina et al., 1996). Listeriosis can cause still births, spontaneous abortion, or even infection in the newborn child from the infected mother (Seeliger and Finger, 1983). The severity of the symptoms depends greatly on the condition of the person infected. Most healthy people develop mild symptoms; however, those with weakened immune systems such as, pregnant, elderly, cancer patients, diabetics, AIDS patients, or persons with kidney disease are more susceptible to the illness (CDC, 2000). In general, mortality rates for listeriosis may be as high as 80% for neonatal infections, and 50-70% for meningitis and septicemia patients (CFSAN, 2000). It has been estimated that *L.*

monocytogenes has caused 2500 cases of illnesses and 500 deaths in the United States each year (CDC, 2000). Patients with listeriosis can be successfully treated with penicillin or ampicillin (CDC, 2000).

The bacterium can come from a variety of sources; however, the primary sources are usually contaminated food for both epidemics and sporadic cases. Thus, the main site of infection is the gastrointestinal tract. The development of the disease and symptoms depends on the size of the inoculum, virulence of the strain, and susceptibility of the person. The incubation period for listeriosis can vary from days to weeks. The minimum amount of bacteria required to cause infection is influenced by numerous variables such as virulence of the bacteria, immune status of the host, type of food consumed, amount of food consumed, and the concentration of bacteria in the food. Currently the minimum infectious dose of *L. monocytogenes* is unknown due to the numerous variables that can affect the value (NACMCF, 1991).

Foodborne Outbreaks

There are several kinds of food that are easily contaminated by *L. monocytogenes*, these foods can include ready to eat (RTE) deli meats, cheeses, meats, dairy products, frankfurters, and seafood. Some possible sources of *L. monocytogenes* are food ingredients, processing aids, contact surfaces for foods, surfaces that do not contact foods, and processing plant environments (Tompkin et al., 1999; USFDA/FSIS, 2003). In the 1980's 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 *L. monocytogenes* outbreaks to establish a "zero tolerance" policy and the FSIS (2003) published a rule that required establishments to develop effective ways to control the bacterium in numerous foods due to a very high risk associated with their consumption (Anonymous, 2003; Swaminathan et al., 2007); however, it is

challenging to completely eliminate the bacterium from foods. In August 1998, 40 illnesses due to high environmental levels of *L. monocytogenes* in the production facility were linked to deli meats and frankfurters (CDC, 2000). Also deli turkey meat was discovered to be responsible for 29 cases of listeriosis in 10 states between May 17 and November 26, 2000 (CDC, 2000). In 2008 there was also an outbreak associated with *L. monocytogenes* contaminating various Maple Leaf deli meats; there were a total of 57 cases of listeriosis reported and 22 deaths associated with the outbreak (CFIA, 2009; Picard, 2008).

Escherichia coli

History

Escherichia coli was originally called *Bacterium coli* commune; the bacteria was first discovered in 1885 by the German pediatrician, Theodor Escherich (Escherich, 1885; Neill et al., 1994). The non-pathogenic form of the bacterium is a normal inhabitant of the intestines of all animals, including humans (FDA, 2001). Most strains of *E. coli* are considered harmless; however, some strains are pathogenic and can cause serious illness. These pathogenic or diarrheagenic (cause diarrheal illness) *E. coli* can be broken up into six classes, which are enteropathogenic (EPEC); enterotoxigenic (ETEC); enteroinvasive (EIEC); diffusely adhering (DAEC); enteroaggregative (EAEC); and enterohemorrhagic (EHEC) (Matthews, 2005). One of the most well known strains, *E. coli* O157: H7, belongs to the EHEC group. *E. coli* O157 was first discovered in 1982 and identified as a causative agent of bloody diarrhea (hemorrhagic colitis, HC) and hemolytic uremic syndrome (HUS) in humans and associated with the consumption of undercooked beef.

Morphology

E. coli is a gram-negative rod-shaped facultative anaerobe. The bacterium is typically found in the gastro-intestinal tract of numerous animals; it is thought that the bacterium inhabits the mucous layer of the mammalian colon where it utilizes gluconate more efficiently than other resident species, thereby occupying a highly specialized niche (Kaper et al., 2004). The bacterium is classified based upon two types of surface structures, which are the O and H antigens. The O antigen refers to the LPS carbohydrate moieties and the H antigen is the flagellar antigen (Besser et al., 1999). *E. coli* O157:H7 is different from other strains of *E. coli* because of its inability to ferment sorbitol rapidly as well as its inability to produce the enzyme β-glucuronidase (Besser, et al., 1999). When the bacterium is grown on violet red bile agar, which contains neutral red pH indicator, the lactose fermentation results in formation of pink colonies.

Growth Requirements

Growth of *E. coli* can occur at 8°C, a temperature to which RTE meals and lightly processed salad vegetables may be exposed for several hours during marketing, transportation or on restaurant buffet counters (Abdul-Raouf et al., 1993). In addition, *E. coli* O157: H7 is unusually tolerant of acidic environments and is capable of growing at a minimum pH of 4.0 to 4.5 (Matthews, 2005). The bacterium can grow in salt levels as high as 6.5% sodium chloride (Sutherland et al., 1995).

Human Illness

E. coli O157:H7 infection most commonly causes symptoms such as abdominal pain, watery diarrhea, bloody diarrhea (hemorrhagic colitis), vomiting and a mild fever (FDA, 2001). Also the bacterium can lead to more severe complications such as hemolytic uremic syndome (HUS) or thrombocytopenic purpura (TTP). The infectious dose of *E. coli* O157:H7 can be as

low as approximately 10 colony forming units (CFUs) (Kothary and Babu, 2001). After ingestion of the organism, there is typically an incubation period of about 3 to 4 days before patients develop diarrhea and for about 25 – 75% of patients the illness remains relatively mild (Besser et al., 1999). The severity of the symptoms depends greatly on the health condition of the person infected. Children under 5 years of age, elderly and, immuno-compromised individuals are in the highest group for infection by this organism. The major illness caused by the bacteria is hemorrhagic colitis, which is characterized by bloody diarrhea, moderate dehydration and acute abdominal cramps. Also HUS occurs in all ages but often in children under 10. The associated symptoms with HUS are pallor, intravascular destruction of red blood cells, depressed platelet counts, lack of urine formation and renal failure. Although the mortality rate of HUS is only 3-5%, many survivors suffer permanent disabilities, such as renal insufficiency and neurological deficits. Commonly in adults TTP occurs, which is similar to HUS except that it causes less renal damage but affects the central nervous system significantly.

The severe virulence of *E. coli* O157:H7 is primarily due to production of verocytotoxins or Shiga-like toxins (SLT), which are most often correlated to a family of bacterial cytotoxins produced by *Shigella dysenteriae* (Sungsu Park et al., 1999). SLT is a protein composed of a single A subunit and five B subunits. The B subunit confers tissue specificity, enabling the toxin to adhere to a specific glycolipid receptor on endothelial cell surfaces. The A subunit is then delivered to the host cell where it binds with 28S ribosome subunits, inhibits protein synthesis, and kills kidney cells, which ultimately leads to HUS.

Foodborne Outbreaks

There are several kinds of food that are easily contaminated by *E. coli*, these foods can include beef, milk, water, various meats, apple juice, sprouts, and vegetables. It is estimated that

there are approximately 63,153 annual domestically acquired episodes of *E. coli* O157:H7 (Scallen et., al 2011). One multi-state outbreak associated with *E. coli* O157:H7 occurred in July 2008. Michigan state health officials tested ground beef purchased by ill persons associated with the outbreak from Kroger retail stores in both Michigan and Ohio (CDC, 2008). It was confirmed from the ground beef tested that the isolates of *E. coli* O157:H7 were the strain involved in the outbreak. There have been 49 confirmed cases linked to this outbreak of which 27 people were hospitalized and one person developed HUS (CDC, 2008). In 1997, there was an outbreak of *E. coli* O157:H7 in Michigan and Virginia associated with alfalfa sprouts (CDC, 1997). A total of 60 people were reported to be affected in Michigan, of those 44 were reported to have bloody diarrhea, 25 were hospitalized, 2 people developed HUS and 1 person had thrombotic thrombocytopenic purpura (CDC, 1997). In Virginia, 48 cases of infection were also diagnosed (CDC, 1997). It was later determined that the alfalfa seeds that were implicated in this outbreak were from a single grower who distributed the contaminated seeds to two farms, one in Michigan and one in Virginia, which produced the contaminated sprouts (CDC, 1997). In 2006 there was a large *E. coli* O157:H7 outbreak involving the consumption of bagged spinach from a plant in California where the contaminated products had been processed (FDA, 2001). It was later determined that the outbreak was associated with Dole baby spinach (FDA, 2001). The direct cause of *E. coli* O157:H7 contamination of the spinach is not clear, but it was thought that the produce was grown near a field which had wild pigs, irrigation wells used to irrigate produce for RTE packaging, and surface waterways exposed to feces from cattle and wildlife (FDA, 2001). The outbreak resulted in 205 confirmed illnesses and 3 deaths (FDA, 2001).

***Salmonella* Typhimurium**

History

Salmonella was named after American veterinary pathologist, Daniel Salmon; however, it was first discovered in 1885 by his assistant Theobald Smith. A subspecies of *Salmonella* isolated from pigs was described in 1885 (Smith, 1894). Since its first discovery the *Salmonella* genus has grown to include 2463 serovars under two species, *Salmonella enterica* and *Salmonella bongori* (Wang, 2006). The serotypes commonly isolated from humans, agricultural products, and foods belong to the subspecies *enteric,* which includes the *Salmonella* Typhimurium serovar. One of the types of *Salmonella* that has become of great concern lately is *S.* Typhimurium DT104. This strain of the bacteria was first identified and characterized in the United Kingdom in the early 1990s and is important because it has chromosomally encoded resistance to ampicillin, chloramphenicol, streptomycin/spectinomycin, sulfonamides, and tetracyclines (ACSSpSuT) (Threlfall, 2000).

Morphology

Salmonella are gram-negative, facultative anaerobic, rod-shaped, non-spore-forming, motile bacteria. These bacteria are oxidase-negative, do not hydrolyze urea, do not produce indole, and are unable to deaminate phenylalanine to tryptophan (Anderson and Ziprin, 1994). The ability of *Salmonella* to produce abundant hydrogen sulphide, decarboxylate lysine, arginine and ornithine and the bacterium's inability to ferment lactose have been utilized in various selective and differential media such as XLD (xylose lysine decarboxylase), RA (Rambach agar), SS agar (Salmonella-Shigella), BGA (brilliant green agar), BGS (brilliant green sulphite agar), MacConkey's agar (Anderson and Ziprin, 1994).The two major species, *Salmonella enterica* and *Salmonella bongori*, are classified according to biochemical

characteristics and the immune-reactivity of two surface structures, the O and H antigens. The O antigen represents a *Salmonella* specific polysaccharide, and the H antigen represents the filamentous portion of the bacterial flagella. Variations on these structures results in different classification within the *Salmonella* genus.

Growth Requirements

Salmonella can grow in temperatures from 2 to 47°C, with the optimum being 25 to 43°C. Most of them can ferment glucose and certain other monosaccharides, but generally cannot ferment lactose, sucrose, or salicin. The bacteria can be killed under high salt concentrations (> 8%) (Wang, 2006). *Salmonella* can also grow best in a moderate pH range of 6.5 – 7.5, high water activity conditions of above 0.94 and can catabolize carbohydrates into acids/gases using citrate as a carbon source (D'Aoust, 2000; Le Minor, 1991; Anderson and Ziprin, 1994). Also the bacteria requires simple inorganic salts containing nitrogen, sulphur, phosphorus and an organic source of carbon and energy for sustaining growth and biochemical reactions (Le Minor, 1991).

Human Salmonellosis

The illness linked to *Salmonella*, salmonellosis, is usually linked to two main manifestations, typhoid or typhoid-like fever and gastroenteritis. The symptoms linked to human salmonellosis include enteric fever, enterocolitis, and invasive systematic disease. Enteric fever is caused by *Salmonella* Typhi and *Salmonella* Paratyphi A, B, or C. The main symptoms consist of watery diarrhea, prolonged fever, nausea, and abdominal cramps. The symptoms of enterocolitis are severe abdominal pain, diarrhea, vomiting and fever. All serotypes of *Salmonella* are potentially pathogenic to humans. Salmonellosis is typically a foodborne illness acquired from contaminated raw poultry, eggs, unpasteurized milk, human-to-human and direct

animal-to-human transmission (Prost, 1967). The illness is especially problematic for newborns, the elderly, and patients with immune deficiencies (D'Aoust, 2001). The onset of human salmonellosis depends on the ability of the bacteria to survive the environment outside the digestive system and the gastric acid of the human stomach and the ability to attach (colonize) and enter (invade) intestinal cells. In general, the infectious dose of bacteria is approximately 10^5 CFUs; however, outbreaks may occur from consumption of relatively low numbers of *Salmonella* cells. Recent evidence suggests that a single cell of *Salmonella* may constitute an infectious dose for humans (D'Aoust, 1997).

Food-borne Outbreaks

Salmonella can be found contaminating numerous foods, such as poultry, dairy-related foods, eggs, sprouts (Taormina et al., 1999), peanut butter (CDC, 2009), tomatoes (Voetsch, 2004), produce, beef, and pork products. Nontyphoidal *Salmonella* accounts for approximately 1 million illnesses, 19, 000 hospitalizations and 400 deaths per year in the U.S (Scallen et al., 2011). One major outbreak occurred in 2009 due to peanut butter and related products infected with *S.* Typhimurium. This peanut butter outbreak involved 714 people from 46 states in the United States (CDC, 2009). Another large outbreak occurred in 2010 due to eggs contaminated with *Salmonella* Enteritidis. This incident led to 1939 cases of illness being reported (CDC, 2010). Also the 2009-2010 outbreak of *Salmonella* Montevideo caused several illnesses. This outbreak occurred when the bacterium contaminated products containing black and red pepper, including Italian-style meat products. This outbreak lead to 272 persons becoming ill in 44 states (CDC, 2010).

Edible coatings

Several non-thermal technologies have been studied to reduce the number of food-borne bacterial outbreaks and illnesses, including the use of antimicrobial edible coatings. In some applications, antimicrobials are suspended in a carrier in order to apply the antimicrobial to the food product. Characteristics of several polysaccharides such as starch, alginate, chitosan, and pectin have been studied in regards to antimicrobial coatings (Nisperos-Carriedo, 1992). In general, because of their hydrophilic nature, high moisture gelatinous polysaccharide coatings can retard moisture loss from food and therefore act as a sacrificing agent (Kester and Fennena, 1986).

Starch and starch derivatives lack active surfaces and thus have to be chemically modified or used in conjunction with emulsifying agents in order to encapsulate hydrophobic products. In an antimicrobial coating the starch acts as a binder for chemical/antimicrobials and therefore helps to extend the product shelf-life. Starch-based coatings can be applied to foods as a smooth, glossy, and fast-drying carrier.

Another carrier commonly used is alginate. The non-hazardous odorless hygroscopic powder is water-soluble and forms viscous solutions. Alginate will form a cold water gel when it is reacted with calcium ions. The substance has been shown to be a desirable a carrier of antimicrobials because of its forming properties, appearance, and its consistency (Joerger, 2007; Coma, 2008). Also when applied to a food matrix the carrier displays very few detectable sensory changes (Juck et al., 2010).

Pectin is a water-soluble hygroscopic polymer that is used as a thickening agent, coating and encapsulating material, in food products. Also pectin is usually naturally found in

the non-woody portions of plants. The substance has been shown to be able to carry and deliver a variety of bioactive substances (Liu et al., 2006).

Another possible carrier for antimicrobials is carrageenan. There are three forms of carrageenan, Kappa, Iota, and Lambda, each with different properties. κ-carrageenan is a hot-water soluble hygroscopic polymer extracted from red seaweed that is often used as a thickening agent or gel in baking and cooking. In previous studies the Kappa (κ) form has been used as a carrier for various antimicrobials (Juck et al., 2010).

Xanthan gum is a hygroscopic water-soluble polymer that is often used as a food ingredient especially in dressings. The polymer is derived from the bacterial coat of *Xanthomonas campestris.* Xanthan gum has previously been used as a carrier for not only antimicrobials (Juck et al., 2010), but also to carry other nutrients (Mei et al., 2002).

Also it should be noted that often acetic acid is used in order to help dissolve the carriers and antimicrobials. The acid is a colorless liquid with a pungent odor and a sour taste that could be used to facilitate dissolution of the carrier materials; note however, that a very small amount is used in dissolution and thus sensory qualities are rarely affected. It is a cheap, and generally recognized as safe (GRAS) substance which serves as an excellent solvent for some organic compounds.

Antimicrobials

Edible coatings serve as effective carriers for a wide range of substances including food ingredients, nutrients, and antimicrobials that can extend 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 is usually the typical point of entry for pathogens and the likely location of maximum microbial contamination (Ming et al., 1997; Janes et al., 2002; Coma, 2008). The

practice of incorporating antimicrobial compounds into edible coatings provides a novel way to improve the safety and shelf-life of foods (Cagri et al., 2004). Various antimicrobials have been used with carriers to form antimicrobial edible coatings.

One very common and popular antimicrobial throughout the food industry is nisin. Nisin is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* during the exponential phase of growth (Hurst and Dring, 1968; Buchman et al., 1988). Nisin is a pentacyclic peptide composed of 34 amino acids. The nisin molecule has a net positive charge due to its high proportion of basic amino acids. Nisin usually does not inhibit molds, yeasts, or gram-negative bacteria, but has a wide spectrum of inhibitory activity against spores and gram-positive bacteria (Mattick and Hirsh, 1947; Lipinska, 1977; Hurst, 1981; Klaenhammer, 1988). The inhibitory effect observed on bacteria has to do with nisin's effect on the cytoplasmic membrane of the bacteria. Due to the positively charged structure of nisin it binds to the cell membrane surface, form a pore in the membrane through amino acid interactions with the hydrophobic lipids of the membrane, and ultimately cause cell lysis (Lipinska, 1977). Damage from nisin to the cell can cause several issues such as the dissipation of the membrane potential and pH gradient, which causes a rapid efflux of amino acids, ATP, and ions from the cellular membrane, the disruption of the proton motive force, and loss of cellular biosynthesis (Ray, 1992).

Another common antimicrobial used in the food industry is sodium lactate (SL) which 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). This chemical preservative has been shown to extend a food product's shelf-life by lowering the water activity of the food (Chirife and Fontan, 1980). It has also been observed that with sub optimum growth temperature (DeWit and Rombouts, 1989) and decreased moisture

(Chen and Shelef, 1992) the effect of SL can be enhanced. Several foods, such as sterile comminuted chicken and beef (Shelef and Yang, 1991), cooked ground beef (Harmayani et al., 1993), and minced beef products (McMahon et al., 1999) have been tested to show SL's antimicrobial activity.

Another antimicrobial, sodium diacetate (SD) is approved as a flavoring agent in meat and poultry products at a level of up to 0.25% by weight of the total formulation (FDA, 2000). Sodium diacetate, which contains acetic acid (40%) and sodium acetate, was first proven useful as a mold inhibitor in baked products, then later in mixed poultry feed, ensiled whole kernel corn, and corn silage (Glabe and Maryanski, 1981). Recent studies have shown that the chemical can be useful and effective at inhibiting various bacteria in several different foods, including meat and poultry. The addition of 0.5% SD alone to turkey slurries inoculated with *L. monocytogenes* exhibited a listericidal effect. Additionally, a combination of 0.5% SD and pediocin or SL showed even greater listericidal ability (Schlyter et al., 1993b). Also a study involving a cooked in bag ham demonstrated the anitlisterial effect of SD; and also showed that it could have an even greater effect when combined with other antimicrobials (Stekelenburg and Kant-Muermans, 2001).

Potassium sorbate (PS) is the water-soluble potassium salt of sorbic acid and is GRAS. The antimicrobial and preservative properties of sorbic acid were discovered in the late 1930's (Luck, 1976; 1980; Sofos and Busta, 1981). Since then the chemical preservative has become widely used in several industries such as for foods, animal feeds, pharmaceuticals and cosmetics (Sofos, 1989). The primary inhibitory action of PS is against yeasts and molds; and its effect against bacteria is not as broad, but appears to be very selective. Effective antimicrobial concentrations of sorbates in most foods are in the range of 0.05 to 0.30%. A study by El-

Shenawy and Marth (1988) determined that 0.2% sorbate inhibited or inactivated *L. monocytogenes* in a broth substrate and in a cold-pack cheese food (Ryser and Marth, 1988). Also 1% PS was shown to cause a slightly decreased *L. monocytogenes* presence in two commercial cheese brines (Larson et al., 1999).

NovaGARD™ CB1 is an antimicrobial blend of substances produced by Danisco Specialties. This antimicrobial blend is a powder composed of maltodextrin, cultured dextrose, SD, sodium chloride, egg white lysozyme and nisin. This antimicrobial combination retards the 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. When used in combination with heat processing, pH, and other hurdles, NovaGARD may delay or prevent growth of selected spore-forming and gram-positive bacterial strains. The application areas of the antimicrobial include deli salads such as chicken, tuna, seafood, ham and RTE meals (Danisco Safety Sheets).

Guardian™ NR 100 is another antimicrobial blend produced by Danisco Specialties. Guardian NR 100 is a powdered blend of nisin and rosemary (Rosmarinus officinalis) extract, which has been shown to exhibit antioxidant properties. This antimicrobial mixture is active against gram-positive bacteria, extends self-life, and enhances product quality. Guardian NR 100 is effective on a variety of food products including low-pH processed meat products, chilled, pasteurized RTE meals, pasteurized soups, and sauces. Also it should be noted that the antimicrobial is effective across a wide range of pH levels (3.5-8.0). The recommended dosage is in the range of 200-500 ppm. Also according to the manufacturer the antimicrobial is composed of 75% (w/w) sodium chloride, 4% (w/w) phenolic diterpenes and 1.25% nisin (Danisco Safety Sheets).

High Hydrostatic Pressure (HHP) Processing

History

HHP processing or high pressure processing is not a new technology; however, its use for food processing to ensure food safety and quality is novel. In 1899 Bert Hite crafted a hydrostatic press that he used to study the effects of high pressure on microorganisms (Hite 1899). However, until the 1980's the use of pressure to reduce bacteria or inhibit their growth was not pursued. In the 1980's R. Hayashi in Japan and D.F. Farkas at the University of Delaware affirmed that high pressure processing decreased microbiological loads and the enzymatic activity in food products while maintaining many of the natural sensory qualities of foods; thus peaking interest in the technology for commercial applications (Garriga et al., 2004). The Japanese market was one of the first areas to have high pressure treated products commercially available such as pressure-processed fruit preserves, jams, and jellies (Hoover, 1997). Since the 1980's and 1990's several foods have been treated with high pressure successfully and become commercially available. Pressure-treated guacamole by Avomex was one of the first very successful and popular pressure stories; it has done very well commercially since its U.S. introduction in 2000. Also oysters are another commercial high pressure success story since the Gold Band™ whole raw oysters are effectively pressure treated to inactivate pathogenic *Vibrio*, parasites, other pathogens, and shuck the oyster.

Mode of Action

High pressure processing is dependent on two main principles, the Principle of Le Chatelier and the Isostatic principle. The Principle of Le Chatelier states that any phenomenon (phase transition, chemical reactivity, change in molecular configuration or chemical reaction) accompanied by a decrease in volume will be enhanced by pressure, and the opposite is true

(Smelt, 1998). Pressure affects reaction systems by reducing the available molecular space and enhancing the inter-chain reactions (Hoover et al., 1989). Thus, HHP is known to have an effect on reactions in biological systems that involve a volume change and will positively influence those reactions that result in volume decrease. The Isostatic Principle states that pressure is transmitted uniformly and immediately through a pressure-transferring medium (Denys et al., 2000). Thus pressure is instantaneously and evenly transmitted through the pressure chamber (Smelt, 1998).

Another important concept that pertains to high pressure processing is adiabatic compression/heating. For many foods water is the main ingredient, and thus the compression of these foods exhibits adiabatic temperature changes very similar to that of their main component. The temperature change of water at room temperature due to adiabatic compression is approximately 3°C for every 100 MPa (Ting et al., 2002). It is known that compressible substances increase in temperature during physical compression and temperature decreases with pressure release (Ting et al., 2002). Thus when high pressure is applied to any food it will increase that foods temperature, the extent of temperature increase depends on the properties of the food. The extent of the temperature increase is also dependent on the amount of pressure applied; the higher the pressure levels, the greater the temperature increase. The inactivation rate of pressure on foodborne pathogens and spoilage microorganisms is increased at temperatures above ambient (U. S. FDA, 2000). Also it has been shown that the temperature at which the food is pressurized can affect the rate of microbial inactivation (Patterson, 2005; Moussa et al., 2006). Finally, it is also possible to achieve greater microbial activation when using high pressure processing with other hurdle technologies such as antimicrobials (Black et al., 2005).

High pressure disrupts the secondary and tertiary structures of macromolecules, such as proteins or polysaccharides, which alters structural and functional integrity (Kalchayanand et al., 1998). Thus cellular mechanisms that allow for repair and replacement of damaged macromolecules increases an organism's tolerance to high pressure. For example, the synthesis of proteins that protect against a range of conditions, such as high salt concentrations, elevated temperatures, and oxidative stress can also increase pressure tolerance (Hill et al., 2002). In *E. coli* and *L. monocytogenes* elevated levels of RpoS and SigB, respectively, increase pressure tolerance (Robey et al., 2001; Wemekamp-Kamphuis et al., 2004). Overall, inactivation of microorganisms by high pressure processing is the result of a combination of factors; cell death is due to multiple damages inside the cell (Simpson and Gilmour, 1997). It has also been reported that high pressure processing can narrow the pH range that a microorganism can withstand by affecting membrane ATPase (Hoover et al., 1989). The effects of high pressure on microorganisms are complex and affect several biochemical processes, such as motility, substrate transport, cell division, growth, DNA replication, translation, transcription, and viability (Bartlette, 2002).

Effect of High Hydrostatic Pressure on Microorganisms

The effect of high pressure processing on a microorganism varies depending on the microorganism itself; some organisms can repair pressure damage more effectively than other organisms (Hoover, 1993). Variation in the inactivation of microorganisms due to stress (pressure in this case) is common, not only between different species but also within species (Simpson and Gilmour, 1997). The difference in pressure resistance was observed in a study by Alpas et al. (1999). It was reported that a 5-min treatment at 345 MPa and 50°C reduced the number *of L. monocytogenes, E. coli* O157:H7, and *Salmonella* by more than 8 log units.

However, a resistant strain of *S. aureus* was not completely inactivated with similar treatment; a 15-min treatment at this pressure resulted in a reduction of only 6.3 log units.

As stated before, higher pressure results in different degrees of microbial inactivation. Pressure treatments between 30 and 50 MPa can influence gene expression and protein synthesis (Hamada et al., 1992). Other studies have shown that at pressures of ~100 MPa the nuclear membrane of yeasts is affected and 400 MPa causes further alteration in the mitochondria and cytoplasm (Hamada et al., 1992). At pressure levels over 300 MPa metal ions are released in microorganisms (Shimada et al., 1993).

Also, the type of medium (food) the organism is in can affect the effectiveness of the pressure treatment. Kalchayanand et al. (1998) found that *L. monocytogenes* Scott A in 0.1 % peptone was reduced by 6.1 log units after a 15-min treatment with 345 MPa at 25°C. However, another study did not observe complete inactivation of Scott A in phosphate-buffered saline modified with bovine serum albumin, glucose, and olive oil with a treatment of 30-min at 450 MPa and at ambient temperature (Simpson and Gilmour, 1997).

The temperature at which the bacteria are pressure treated can also effect the reduction of the microorganisms. Increasing the treatment temperature above 30°C will generally increase the effectiveness of the HHP treatment (Cheftel**,** 1995; Roberts and Hoover, 1996). Ogawa et al. (1990) observed an increase in the inactivation of naturally present as well as artificially contaminated microorganisms in mandarin juice treated at 40°C (as compared to lower temperatures) at pressures ranging from 400-450 MPa.

The growth phase of the bacteria being treated can also affect the outcome of pressure treatments. Generally it seems that stationary phase cells are more pressure resistant than exponential phase bacteria (Mañas and Mackey, 2002). McClement et al., (2001) found that

exponential-phase cells were less resistant to pressure than stationary-phase cells for all of the three species studied. Also growth temperature was found to have a significant effect at the two growth stages studied. For *L. monocytogenes* (NCTC and Scott A), *Bacillus cereus* (NCFB 578 and 1031), *Pseudomonas fluorescens* (NCDO 1524) exponential cells grown at 8°C were more resistant than those grown at 30°C , but for stationary-phase cells the reverse was true. In another study by Pagan and Mackey (2000) it was observed that loss of viability was correlated with a permanent loss of membrane integrity in logarithmic-growth phase cells, whereas injured membranes of stationary-phase cells were able to repair following pressure treatment. Also it seems that stationary phase cells can synthesize various proteins that protect against stresses such as elevated salinity, high temperatures and oxidative damage (Hill et al., 2002).

Growth temperature can also affect the pressure treatment of bacteria. Typically as growth temperature is increased, it is accompanied by an increase in membrane fluidity (Mañas and Mackey 2002), which in turn affects the degree of pressure sensitivity. The influence of growth temperature on pressure-resistance of bacteria varies depending on the growth phase of the culture. Shearer et al. (2010) observed that *L. monocytogenes* grown at 10, 15, 20 or 25°C, suspended in UHT milk, and pressure-treated at a pressure level of 400 MPa exhibited lowest pressure resistance with ca. 6.5-log CFU/mL population reductions. However, at higher growth temperatures of 30, 35, 40 and 43°C, a progressive increase in pressure resistance was observed.

Also storage or recovery temperature after pressure treatment can affect the degree of inactivation of bacteria. Shearer et al. (2010) reported that maximal recovery of *L. monocytogenes*, inoculated into UHT milk and exposed to pressure-treatment, occurred at a storage temperature of 10°C when tested within the 4 to 40°C range.

Gas atmosphere can also affect the survival of bacteria after pressure treatment. Ye et al. (2011) showed that aerobic incubation promoted greater recovery of pressure-treated cells of *Vibrio parahaemolyticus* inoculated into oyster meats although there was no statistically significant difference in the counts. Work conducted by Bull et al. (2005) indicated that recovery of pressure-injured *L. monocytogenes* in milk occurred equally well or better in aerobic enrichment broth than in anaerobic enrichment broth. Another study by Aertsen et al. (2005) found that when *E. coli* MG1655 was pressure treated at 300 and 400 MPa then stored aerobically and anaerobically, the anaerobic conditions led to improved cellular survival.

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Chapter 3

APPLICATION OF AN ACTIVE ALGINATE COATING TO CONTROL THE GROWTH OF LISTERIA MONOCYTOGENES ON POACHED AND DELI TURKEY PRODUCTS

Abstract

The relatively high prevalence of *Listeria monocytogenes* in ready-to-eat (RTE) turkey products is of great concern. The overall objective of this study was to develop antimicrobial edible coating formulations to effectively control the growth of this pathogen. The antimicrobials studied were nisin (500 IU/g), Novagard CB 1 (0.25%), Guardian NR100 (500 ppm), sodium lactate (SL, 2.4%), sodium diacetate (SD, 0.25%), and potassium sorbate (PS, 0.3%). These were incorporated alone or in binary combinations into five edible coatings: alginate, κ-carrageenan, pectin, xanthan gum, and starch. The coatings were applied onto the surface of home-style poached and processed deli turkey discs inoculated with ~ 3 log CFU/g of *L. monocytogenes*. The turkey samples were then stored at 22°C for 7 days. For poached and processed deli turkey, the coatings were found to be equally effective, with pectin being slightly less effective than the others. The most effective poached turkey treatments were SL (2.4%)/SD (0.25%) and Nisin (500 IU/g)/SL (2.4%), which yielded final populations of 3.0 and 4.9 log CFU/g respectively compared to the control which was 7.9 log CFU/g. For processed deli turkey, the most effective antimicrobial treatments were nisin (500 IU/g)/SD (0.25%) and nisin (500 IU/g)/SL (2.4%) with final populations of 1.5 and 1.7 log CFU/g respectively compared to the control which was 6.5

log CFU/g. In the second phase of the study, home-style poached and store-purchased roasted (deli) turkey inoculated with the pathogen at a level of ~ 3 log CFU/g were coated with alginate incorporating selected antimicrobial combinations and stored for 8 weeks at 4°C. Alginate coatings supplemented with SL (2.4%)/PS (0.3%) delayed the growth of *L. monocytogenes* with final counts reaching 4.3 log CFU/g (home-style poached turkey) and 6.5 log CFU/g (roasted deli turkey), respectively, while the counts in their untreated counterparts were significantly higher ($P < 0.05$) reaching 9.9 and 7.9 log CFU/g, respectively. This study therefore demonstrates the effectiveness of using alginate-based antimicrobial coatings to enhance the microbiological safety and quality of RTE poultry products during chilled storage.

Introduction

Listeria monocytogenes is a serious safety concern for the food industry, especially with respect to ready-to-eat (RTE) meat, dairy, and seafood products. *L. monocytogenes*, a gram-positive, non-spore-forming rod-shaped bacterium, is responsible for the foodborne illness listeriosis. It is estimated that in the United States, there are annually 2500 cases of illness and 500 deaths due to listeriosis (Lungu and Johnson, 2005). The symptoms of listeriosis are fairly variable and range from a mild-flu like illness to more serious complications such as meningitis, septicemia, stillbirths and abortions (Salamina et al., 1996). In general, the major symptoms of disease are restricted to pregnant women, neonates, the elderly and people with weak immune systems with a reported high mortality rate (McLauchlin, 1997).

A variety of RTE food products have been implicated in outbreaks of *L. monocytogenes*. The ubiquitous nature of *L. monocytogenes* can be attributed to its growth characteristics. *L. monocytogenes* can tolerate a high salt level of up to 16%; it is psychrotrophic with a minimum growth temperature of -0.4°C; it has a wide pH range of 4.2-9.6 and is facultatively anaerobic

(Farber, 1991). These growth requirements are easily met by numerous food groups such as RTE poultry products including cooked turkey which is characterized by a salt content of < 2%, a pH of about 6.5, a storage temperature of around 4°C and a vacuum packaging environment. In addition, *L. monocytogenes* is known to be able to re-contaminate cooked meat or poultry products during the processing and packaging steps (Janes et al., 2002).

Various methods have been proposed to control post-process contamination of RTE meat and poultry products by *L. monocytogenes*. Use of thermal processing such as hot water, steam and radiant heat to inactivate *L. monocytogenes* in RTE meat and poultry have been studied (Ingham et al., 2005; Murphy et al., 2002; 2003a; 2003b; 2003c; 2005; Muriana et al., 2002; 2004). Others have investigated nonthermal processing methods such as high pressure processing (Chen, 2007) and irradiation (Zhu et al., 2005) to ensure the safety of various RTE meats. Other studies have been conducted using modified atmosphere packaging (MAP) in order to inhibit the growth of *L. monocytogenes*; often MAP is coupled with other technologies such as irradiation on turkey meat (Thayer and Boyd, 1999) or antimicrobials on cooked pork (Fang and Lin, 1994). The inclusion of antimicrobials into meat formulations to inhibit the growth of *L. monocytogenes* has also been reported (Barmpalia et al., 2004, 2005). In this study the overall objective was to develop effective antimicrobial edible coatings to control the growth of *L. monocytogenes* on several turkey products formulated or processed differently.

Materials and Methods

2.1. Identification of a most effective antimicrobial edible coating material and treatments at room temperature

2.1.1 Bacterial strains and culture conditions

Five *L. monocytogenes* strains, PSU1, PSU9, F5069, ATCC 19115 and Scott A were

used. The strains were maintained on tryptic soy agar plus 0.6% yeast extract (TSAYE) (Difco Laboratories, Sparks, MD) plates and stored at 4°C. Each strain was grown separately in tryptic soy broth with yeast extract (TSBYE) for 24 h at 35°C and 100 μl of each overnight culture was transferred to fresh TSBYE broths for another 24-h incubation. On the day of the experiment, a 1-ml volume of each culture was combined to provide a five-strain composite and then readjusted with 0.1% peptone water to cell densities of ca. 10^5 CFU/ml, which served as the inoculum. Serial dilutions were plated onto TSAYE plates and incubated at 35°C for 24 h to determine initial cell numbers.

2.1.2 Inoculation and treatment of turkey samples stored at room temperature

Raw sliced turkey and processed deli turkey were obtained from a local grocery store, kept frozen at -20°C and thawed at 4 ± 1°C for 1 day immediately before use. The raw turkey was punched into 5.7-cm diameter round pieces weighing 22 ± 1 g, which were then placed into 3 mm thick high barrier pouches (nylon/polyethylene, Koch Supplies, Kansas City, MO) before being sealed using a vacuum-packaging machine (Model Ultravac 225 with digital control panel, Koch Equipment, Kansas City, MO). The turkey samples were then poached in a 90°C circulating water bath for 10 min and immediately cooled in chilled water. The processed deli turkey were punched into 5.7-cm diameter round pieces weighing 7 ± 1 g. The turkey sample weights differed between the turkey types because the poached turkey was thicker than the processed turkey. The processed deli meat had a pH of 6.4, a water activity of 0.928, and its main ingredients were: turkey, water, modified corn starch, less than 2% of corn syrup, sodium lactate (SL), salt dextrose, flavor, sodium phosphates, sodium diacetate (SD), sodium ascorbate, and sodium nitrate. The salt content of the deli turkey was approximately 1%. The poached turkey had a pH of 6.1 and a water activity of 0.929. Since the poached turkey was made from

fresh raw turkey it would be expected to have a negligible salt content. The poached and processed turkey samples were surface-inoculated with aliquots of 110 and 35 µl respectively, of a 10^5 CFU/ml dilution of the five-strain cocktail of *L. monocytogenes* on each side to achieve a final concentration of ~10^3 CFU/g.

Five polysaccharides, alginate (TIC Gums, MD, USA), κ-carrageenan (TIC Gums), pectin (TIC Gums), starch (Instant Pure-Cote® B792, Grain Processing Corporation, IA, USA) and xanthan gum (TIC Gums), were compared for their suitability as carriers of antimicrobials when applied as an edible coating on turkey products. The coating solutions were prepared by mixing 2.4 g of alginate, 2 g of pectin, 2 g of κ-carrageenan, 30 g of starch or 2 g of xanthan gum in 200 ml of a 1% acetic acid solution with overnight stirring at room temperature (22°C) (Neetoo et al., 2009). A preliminary study performed in our laboratory showed that there was no significant antimicrobial activity due to the 1% acetic acid in the coatings (unpublished data). Coating solution were then supplemented with SL (Purac America Inc., IL, U.S.A), SD (Purac), potassium sorbate (PS) (Alfa Aesar), nisin (Nisaplin, Danisco Specialties), Novagard CB 1 (Danisco Specialties) and Guardian NR100 (Danisco Specialties). Guardian NR 100 is an antimicrobial system with active ingredients consisting of nisin and rosemary extract. The active ingredients of Novagard CB 1 are egg white lysozyme, nisin, and sodium diacetate, and sodium chloride. The pH of each of the coatings and treatments was measured using a pH meter (model pHTestr 20, EuTech Instruments and Oakton Instruments, Vernon Hills, IL). Aliquots of 210 and 660 µl of each coating solution containing different antimicrobials were then applied onto each side of the processed and poached turkey discs respectively, to obtain the levels of antimicrobials desired for each treatment. Samples were allowed to dry by leaving them in a laminar-flow hood under ventilation for about 20 minutes after the coating was applied on each

side, with a total drying time of 45 minutes. Controls, inoculated samples without coating treatments, were also prepared. The controls and treated inoculated turkey samples were then vacuum-packaged as described above and stored at 22°C.

2.1.3 Microbial enumeration of inoculated samples

After 7 days of storage, processed and poached turkey samples were individually placed in stomacher bags containing 28 and 100 ml of 0.1% sterile peptone water respectively, and stomached with a Seward 400 Stomacher (Seward Medical Company, London, England) at 260 rpm for 2 min. The amount of peptone water added to the turkey meat in the stomacher bag was relative to the weight of the turkey meat; the ratio of sample to peptone water was 1:4. Serial dilutions were made in 0.1% peptone water, and counts of *L. monocytogenes* were determined by an overlay method (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 Laboratories) at 45°C was overlaid on the TSAYE plates. The plates were incubated at 35°C for 48 h and small black colonies with black haloes on the plates were counted.

2.2. Determining the effectiveness of an alginate-based antimicrobial coating in controlling L. monocytogenes and spoilage microorganisms on poached and roasted deli turkey

Alginate-based antimicrobial treatments demonstrating satisfactory anti-listerial activity were selected and further investigated for their long-term effectiveness on poached and roasted deli turkey. Poached turkey slices were prepared as described above. Roasted turkey was the deli meat of choice in this part of the study since it did not contain any added antimicrobials, ensuring that results obtained only reflected the effect of the antimicrobials being tested, as opposed to the

processed deli turkey that was formulated with certain compounds that could potentially have an antilisterial effect. Roasted turkey slices, obtained from a local grocery store, were punched into 5.7-cm diameter discs weighing 31 ± 3 g. The roasted turkey had a pH of 6.1 and a water activity of 0.933, and its main ingredients were: roasted turkey meat, turkey broth, salt, sugar, and paprika. The preparation of the five-strain cocktail of *L. monocytogenes* and surface-inoculation of poached and roasted turkey samples was carried out as described above. Each side of the turkey discs were subsequently coated with a 660 (poached) or 930 (roasted deli) μl aliquot of alginate solution containing binary combinations of SL (2.4% w/w), SD (0.25% w/w), PS (0.3%), nisin (500 IU/g), Novagard CB 1 (0.25%), or Guardian NR100 (500 ppm). Coated samples were 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 as the ones used for the inoculated samples. All the samples were then vacuum packaged and stored at 4°C for up to 8 weeks. Inoculated samples were microbiologically analyzed for *L. monocytogenes* counts every 7 days. Un-inoculated samples were analyzed for aerobic and anaerobic counts every 14 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. Aerobic bacteria counts were determined by plating onto TSAYE plates and incubated aerobically at 35°C for 2 days.

2.3. Statistical analysis

Three independent trials were conducted for all the experiments. Where appropriate, statistical analyses were conducted using JMP® 8.0.1, a statistical analysis program developed by SAS (SAS Institute Inc.,Cary, NC, USA). One-way analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were used to determine differences in the populations of *L.*

monocytogenes, aerobes and anaerobes on turkey samples. Significant differences were considered at the 95% confidence level ($P < 0.05$).

Results

3.1 Effect of various polysaccharide-based edible coatings on the growth of L. monocytogenes on home-style poached and processed deli turkey stored at room temperature

The pH values for all the treatments and five coating types are shown in Table 1. Typically these antimicrobials would be expected to be more effective in a more acidic environment. The antilisterial effects of the various antimicrobial edible coatings as applied onto home-style poached and processed turkey slices after a seven-day storage period are presented in Tables 2 and 3, respectively. The inhibitory activity of the various edible coatings was found to be dependent mainly on the nature of the antimicrobial(s) incorporated, the level of antimicrobial(s) used, and the type of binary combinations. The counts of *L. monocytogenes* present on untreated controls of poached and deli turkey rose to a maximum of 7.9 and 6.5 log CFU/g, respectively, over a period of 7 days storage at room temperature. After 7 days, all samples treated with antimicrobial-containing coatings produced lower counts of *L. monocytogenes* compared to the untreated control. The most effective antimicrobial treatments applied for the fresh poached turkey ranked in the order of SL (2.4%)/ SD (0.25%) > nisin (500 IU/g)/SL (2.4%) > nisin (500 IU/g)/SD (0.25%). The most effective antimicrobial treatments applied for the processed deli turkey ranked in the order of nisin (500 IU/g)/SL (2.4%) > nisin (500 IU/g)/SD (0.25%) > SL (2.4%)/SD (0.25%). Binary combinations of SL and/or SD and/or nisin recurrently appeared as the most effective treatments for both turkey products. The edible coatings incorporating antimicrobials were significantly more effective on the deli turkey meat than on poached turkey ($P < 0.05$). The different antimicrobial treatments had comparable

efficacy across the various coatings, although pectin was slightly less effective than the others (Table 1). Therefore, one of the four more promising coating types, alginate, was chosen for the subsequent refrigerated study.

3.2 Effectiveness of an alginate-based antimicrobial coating in controlling L. monocytogenes and spoilage microorganisms on poached turkey at 4^{o}C

The populations reached after 8 weeks of storage for poached turkey samples treated with alginate coating incorporating various antimicrobials are shown in Table 4. *L. monocytogenes* on inoculated poached turkey grew very rapidly, reaching 7.1 log CFU/g after 2-week storage and 9.9 log CFU/g after 8-week storage. The growth of *L. monocytogenes* on the treated turkey samples were inhibited depending on the treatments, reaching between 4.3 and 8.5 log CFU/g by the end of the storage time. The three best treatments ranked in the order of decreasing effectiveness were SL (2.4%)/PS (0.3%) > nisin (500 IU/g)/SD (0.25%) > nisin (500 IU/g)/SL (2.4%). We only observed a marginal growth of *L. monocytogenes* (< 1 log increase in counts) during the entire storage period with the most effective treatment SL (2.4%)/PS (0.3%), resulting in a final population of 4.3 log CFU/g.

The aerobic microorganisms for un-inoculated poached turkey in the control samples grew very rapidly, increasing by 3.0 log CFU/g after only 2 weeks of storage and by 6.2 log CFU/g after 8 weeks of storage (Table 5). Antimicrobial treatments slowed down their growth to varying degrees. Of all the antimicrobial combinations applied, the most effective treatments ranked in the order of decreasing effectiveness were: nisin (500 IU/g)/SD (0.25%) > nisin (500 IU/g)/SL (2.4%) > nisin (500 IU/g)/PS (0.3%). The binary treatment of nisin/SD resulted in an initial population decrease from 2.2 log CFU/g to 1.8 log CFU/g during the second week and eventually increased, reaching a final population of 6.2 log CFU/g. The population of anaerobes

in the control samples increased from an initial population of 1.4 log CFU/g to a maximum of 9.1 log CFU/g, with treated turkey samples reaching between 7.4 and 8.4 log CFU/g by the end of the storage time, as seen in Table 5. Of all the antimicrobial treatments applied the most effective for inhibition the growth of anaerobes were nisin (500 IU/g)/SL (2.4%) $\geq$ SD (0.25%)/PS (0.3%) > nisin (500 IU/g)/SD (0.25%). The most effective treatment (nisin/SL) resulted in a gradual increase in anaerobic counts to 3.5 log CFU/g at 4 weeks and 5.3 log CFU/g at 6 weeks, respectively. Background aerobic and anaerobic microorganisms proliferated rapidly in the control (untreated sample) and resulted in spoilage after 4 weeks, assuming a population of 10^7 CFU/g as the spoilage limit (Hoornstra et al., 2001). On the other hand, all the antimicrobial treatments extended the shelf-life from 4 to 6 weeks.

3.3 Effectiveness of an alginate-based antimicrobial coating in controlling L. monocytogenes and spoilage microorganisms on roasted turkey at 4^{o}C

Table 6 shows the effects of the various alginate-based treatments on the population of *L. monocytogenes* on roasted turkey during 8 weeks of storage at 4°C. The initial population of *L. monocytogenes* on roasted turkey was 3.0 log CFU/g with the population rising to 6.3 log CFU/g after 2-weeks of storage and a final level of 7.9 log CFU/g after 8-weeks of storage in the control sample. The *L. monocytogenes* population of treated samples ranged from 6.5 to 7.8 log CFU/g at the end of storage. Of all the antimicrobial treatments applied, the most effective were in the order of SL (2.4%)/PS (0.3%) > SD (0.25%)/PS (0.3%) > nisin (500 IU/g)/SL (2.4%). The most effective treatment (SL/PS) considerably slowed down the growth of *L. monocytogenes*, resulting in populations of 4.9 log CFU/g and 6.5 log CFU/g at week 6 and week 8, respectively.

Table 7 shows the effects of various alginate-based treatments on the aerobic and anaerobic bacterial populations of un-inoculated roasted turkey during the 8 weeks of storage at

4°C. The aerobic counts increased from the initial level of 1.6 log CFU/g to 6.9 CFU/g at 2 weeks and finally to a maximum of 7.4 log CFU/g in the control samples, while the treated samples produced between 5.7 and 7.1 log CFU/g by the end of the storage period. The three most effective antimicrobial treatments were in the order of SL (2.4%)/PS (0.3%) > Novagard CB1 (0.25%) > nisin (500 IU/g)/PS (0.3%), achieving counts that were 1.7 - 0.8 log CFU/g lower than the control. The anaerobic count increased from an initial population of 1.3 log CFU/g to a maximum of 7.5 log CFU/g in the control samples with treated turkey samples achieving between 6.4 and 7.2 log CFU/g by the end of the storage time. Of all the antimicrobial treatments applied the most effective were SD (0.25%)/PS (0.3%) > nisin (500 IU/g)/PS (0.3%) > SL (2.4%)/PS (0.3%). The most effective treatment (SD/PS) produced a count of 0.5 log CFU/g lower than the control at week 4 and at week 8 resulted in a count of1.1 log CFU/g lower than the control. Aerobic and anaerobic bacteria grew rapidly in the control sample and resulted in spoilage after 2 weeks while the three most effective treatments had a shelf-life of at least 8 weeks, assuming microbial spoilage occurs above a threshold of 7 log CFU/g.

The best antimicrobial formulations for both types of RTE turkey products under refrigerated storage were very similar and also similar to those identified in the room temperature study. These treatments consisted of combinations of SL, SD, and/or nisin in combination. In addition, PS also demonstrated effective anti-listerial ability at refrigeration temperature for both roasted and poached turkey.

Discussion

In the current study, we observed that most of the coating types appeared to be generally equally effective, with the exception of pectin, which showed less of an inhibitory effect. Alginate, pectin, carrageenan, and starch coatings were also previously shown to enhance the

antilisterial activity of incorporated antimicrobials when applied onto cold-smoked salmon (Neetoo et al., 2009). Datta et al. (2008) also demonstrated the antilisterial efficacy of a calcium alginate coating incorporating nisin on smoked salmon. Xanthan gum has previously been shown to improve the sensory and nutritional qualities of carrots (Mei et. al, 2006). Results in Tables 2 and 3 show that the coating treatments were significantly more effective against *L. monocytogenes* when used for the processed deli turkey than for the poached turkey ($P < 0.05$). One possible reason to explain this difference could be the presence of antimicrobials such as salts of organic acids that had been added to the deli meat by the processor.

It can be observed from Tables 2 and 3 that SL, SD and nisin were components of the most effective binary combinations (SL (2.4%)/SD (0.25%) and nisin (500 IU/g)/SL (2.4%)) for both poached and processed turkey. SL is approved as a Generally Recognized as Safe (GRAS) substance and allowed at 4.8% (U.S. FDA, 2000). SL has been shown to be effective with regard to *L. monocytogenes* inhibition in foods such as ham steaks, comminuted chicken and beef model systems, cook-in-bag roasts and cold-smoked salmon (Chen and Shelef, 1992; Shelef and Yang, 1991; Unda et al., 2006; Ye et al., 2008a; 2008b). SL has also been shown to improve not only the flavor, but the processing yields of various meats (Shelef and Addala, 1994). SD is approved as a GRAS substance at a level up to 0.25% by weight of the total formulation (U.S. FDA, 2000). Previous studies have already shown the effectiveness of SD against *L. monocytogenes* in foods such as in turkey slurries (Schlyter et al., 1993), wieners and cooked bratwurst (Glass et al., 2002), and cold-smoked salmon (Neetoo et al., 2008a; Vogel et al., 2006). Nisin, a GRAS antimicrobial polypeptide, is limited to 0.00125% or 500 IU/g for ripened cheese and processed cheese by the European Commission (Neetoo et al., 2008b). Nisin has been shown previously to be effective against gram-positive bacteria and exert an antilisterial effect on foods such as milk

(Bhatti et al., 2004), frankfurters (Luchansky and Call, 2004), cheese (Samelis et al., 2003), pork (Murray and Richard, 1997), salmon (Nilsson et al., 1997; Neetoo et al., 2008a; b) and beef (Avery and Buncic, 1997). The binary combination of nisin with the other two compounds (SL and SD) appeared to provide a synergistic effect since the combination of the two compounds had a greater antilisterial effect than the individual compounds alone. Neetoo et al. (2009) tested SL and SD in combination on cold-smoked salmon and found the combination was effective against *L. monocytogenes*. In addition, we found that PS in combination with SL and SD also displayed satisfactory antilisterial activity on both poached and roasted turkey during extended refrigerated storage as seen in Tables 4 and 6. PS has previously been shown to inhibit *L. monocytogenes* in foods such as turkey (Wederquist et al., 1994), turkey frankfurters (Islam et al., 2002), and commercial cheese brines (Larson et al., 1999). Bari et al. (2005) also previously demonstrated the efficacy of binary combinations of SL and PS against *L. monocytogenes* on cabbage, broccoli, and mung bean sprouts.

Although SL, SD, nisin, and PS were found to be inhibitory to *L. monocytogenes* in roasted turkey, the effect was not as pronounced as in poached turkey (Tables 4 and 6). This could be due to the fact that the roasted turkey was formulated with turkey broth and thus had a higher moisture content while the poached turkey samples did not have broth. The higher moisture content and hence higher water activity would be expected to support greater microbial growth. In addition, table sugar, another ingredient present in roasted turkey meat formulation and absent in poached turkey, could have also promoted faster bacterial growth.

Several of the coating treatments were not only inhibitory to *L. monocytogenes*, but also slowed down the growth of aerobic and anaerobic spoilage bacteria. Nisin, SL, SD, and PS all showed a strong inhibitory effect to aerobic bacteria on poached turkey. It has previously been

shown that nisin is effective against background aerobes in minced pork meat (Soriano et al., 2004), in rainbow trout (Nykänen et al., 2000), and in cantaloupes and on honeydew melons (Nykänen et al., 2005). SL has also been shown to be inhibitory against aerobic bacteria in rainbow trout (Nykänen et al., 2000), on cantaloupes and honeydew melons (Nykänen et al., 2005), and in cold-smoked salmon (Neetoo et al., 2009). SD was also shown in previous experiments to have a strong inhibition to aerobic bacteria on ground beef (Shelef et al., 2007) and cold-smoked salmon (Neetoo et al., 2009). Finally, it has been shown in past studies that PS also has a strong aerobic antimicrobial effect on such foods as Turkish-style sausage (Bozkurt et al., 2002) and shrimp (Mosffer et al., 1999). This study also highlighted the importance of antimicrobials such as nisin, SL, SD, and PS to inhibit anaerobic bacteria on poached turkey. Previously, Roberts et al., (1992) demonstrated that nisin was effective against anaerobic bacteria in cheese. The antimicrobial effect of SL towards anaerobic bacteria was also previously demonstrated in beef (Serdengecti et al., 2006) and in cold-smoked salmon (Neetoo et al., 2009). SD has been reported by other researchers to have a strong inhibition to anaerobic bacteria on beef (Serdengecti et al., 2006) and cold-smoked salmon (Neetoo et al., 2009). Finally, it has been shown in past studies that PS also has a strong antimicrobial effect against anaerobes on such foods as poultry (McMeekin et al., 2007) and emulsified meatballs (Hsu et al., 2006). With regard to roasted turkey, nisin, SL, and PS all displayed a high degree of inhibition to aerobic and anaerobic bacteria on roasted turkey. In addition, Novagard CB 1 was effective in retarding the growth of aerobic bacteria. Other researchers have also demonstrated the effectiveness of Novagard CB1 in combination with other antimicrobials to simultaneously inhibit pathogen proliferation on dairy products such as cheese or milk (U.S. Patent 20080152759) and extend their shelf-life.

Conclusion

This study leads to the general conclusion that alginate-based antimicrobial coatings can improve the safety of poached and roasted turkey by inhibiting the growth of *L. monocytogenes*. Our findings further demonstrated that the incorporation of binary combinations of antimicrobials at final concentrations of SL (2.4%)/SD (0.25%) and nisin (500 IU/g)/SL (2.4%) into the alginate coating, effectively inhibited the growth of *L. monocytogenes* as well as the background microbiota levels throughout an 8-week storage period at 4°C on both RTE turkey product types, especially on poached turkey. Furthermore, it should be noted that all of the coatings throughout the experiments displayed good adherence and stability on the turkey. The edible coatings also did not alter the texture, color, smell, and overall appearance of the final product. Most studies including ours have been carried out as trials in which *L. monocytogenes* was spiked onto the product at a level that may exceed the realistic level of contamination, thus leading to possible underestimation of the efficacy of the treatments. It is likely therefore that these aforementioned surface treatments could be highly viable for use by the RTE poultry industry.

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Tables

Table 1

The pH values for different treatments and coating types before applying to the turkey meats

	Coating Types				
Treatments	Pectin	Alginate	Carrageenan	Starch	Xanthan Gum
Coating only	3.4	3.7	3.0	3.0	3.3
SL (2.4%)	5.5	5.7	5.5	5.4	5.4
SD (0.25%)	4.5	4.6	4.6	4.5	4.5
PS (0.3%)	6.2	6.0	6.1	5.9	6.1
Nisin (500 IU/g)	3.0	3.4	3.1	3.1	3.2
SL (2.4%)/SD (0.25%)	5.3	5.5	5.4	5.2	5.3
SL (2.4%)/PS (0.3%)	6.3	6.4	6.3	6.4	6.4
SD (0.25%)/PS (0.3%)	5.3	6.0	5.2	5.4	5.4
Nisin (500 IU/g)/SL (2.4%)	5.4	5.6	5.5	5.3	5.5
Nisin (500 IU/g)/SD (0.25%)	4.3	4.3	4.4	4.4	4.3
Nisin (500 IU/g)/PS (0.3%)	5.7	5.2	5.8	5.8	5.8
Guardian NR 100 (500 ppm)	3.2	3.2	2.8	3.3	2.9
Novagard CB1 (0.25%)	4.3	4.5	4.4	4.3	4.4

Table 2
Effect of different antimicrobial edible coating treatments on the populations of *L. monocytogenes* on poached turkey stored at room temperature for 7 days. The initial inoculation level was 2.8 log CFU/g. Data are the means of three replicates ± one standard deviation (log CFU/g).

	Coating Types				
Treatments	Pectin	Alginate	Carrageenan	Starch	Xanthan Gum
Coating only	7.8±0.3^{A}	7.8±0.1^{A}	7.7±0.2^{AB}	7.5±0.8^{A}	7.8±0.3^{A}
SL (2.4%)	6.8±0.7^{AB}	6.9±0.9^{AB}	6.1±1.6^{ABC}	6.4±0.7^{ABC}	7.0±0.8^{A}
SD (0.25%)	6.7±0.9^{AB}	6.7±0.7^{ABC}	6.0±1.2^{ABC}	7.0±0.8^{A}	6.7±0.6^{AB}
PS (0.3%)	6.8±0.9^{AB}	7.0±0.8^{AB}	6.9±0.6^{AB}	7.0±1.0^{A}	7.2±0.9^{A}
Nisin (500 IU/g)	6.9±0.9^{AB}	7.1±0.6^{AB}	7.0±0.6^{AB}	7.2±0.5^{A}	7.1±0.9^{A}
SL (2.4%)/SD (0.25%)	4.1±1.4^{C}	3.0±0.7^{D}	3.7±1.1^{C}	4.3±0.9^{BC}	3.7±1.5^{C}
SL (2.4%)/PS (0.3%)	6.5±0.7^{ABC}	5.9±0.4^{ABC}	6.2±1.2^{ABC}	6.3±1.1^{ABC}	6.4±0.3^{AB}
SD (0.25%)/PS (0.3%)	6.4±0.8^{ABC}	6.1±1.1^{ABC}	6.3±1.1^{ABC}	6.4±0.9^{ABC}	6.3±0.4^{AB}
Nisin (500 IU/g)/SL (2.4%)	5.5±1.4^{ABC}	4.9±0.2^{CD}	4.9±0.7^{BC}	4.4±0.3^{BC}	4.3±0.4^{BC}
Nisin (500 IU/g)/SD (0.25%)	4.9±0.3^{BC}	5.2±0.4^{BC}	5.2±0.4^{ABC}	4.0±1.6C	5.7±0.1^{ABC}
Nisin (500 IU/g)/PS (0.3%)	6.9±0.7^{AB}	6.8±1.1^{ABC}	6.9±1.3^{AB}	6.8±1.0^{AB}	5.8±1.1^{ABC}
Guardian NR 100 (500 ppm)	7.4±0.5^{AB}	7.4±0.2^{A}	7.3±0.5^{AB}	7.4±0.5^{A}	7.5±0.5^{A}
Novagard CB1 (0.25%)	7.3±0.8^{AB}	7.3±0.6^{A}	6.5±1.5^{ABC}	7.3±0.5^{A}	7.1±1.0^{A}
Control	7.9±0.3^{A}	7.9±0.3^{A}	7.9±0.3^{A}	7.9±0.3^{A}	7.9±0.3^{A}

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

Table 3
Effect of different antimicrobial edible coating treatments on the populations of *L. monocytogenes* on processed deli turkey stored at room temperature for 7 days. The initial inoculation level was 3.1 log CFU/g. Data are the means of three replicates ± one standard deviation (log CFU/g).

	Coating Types				
Treatments	Pectin	Alginate	Carrageenan	Starch	Xanthan Gum
Coating only	5.4 ± 0.3^{ABa}	5.3 ± 0.5^{ABa}	4.8 ± 0.2^{ABa}	4.2 ± 0.3^{Ba}	5.4 ± 1.1^{ABa}
SL (2.4%)	3.4 ± 0.7^{BCDEa}	2.9 ± 0.2^{CDEa}	2.7 ± 0.5^{BCa}	2.6 ± 0.2^{BCDa}	3.3 ± 0.7^{BCDEa}
SD (0.25%)	2.9 ± 0.0^{CDEa}	2.6 ± 0.6^{CDEa}	2.7 ± 0.5^{BCa}	2.2 ± 0.4^{BCDa}	3.0 ± 0.2^{CDEa}
PS (0.3%)	4.7 ± 0.1^{ABCa}	3.7 ± 0.8^{BCa}	4.1 ± 1.5^{ABCa}	3.2 ± 0.4^{BCDa}	4.7 ± 1.5^{ABCa}
Nisin (500 IU/g)	4.3 ± 1.4^{BCa}	2.8 ± 1.0^{CDEa}	3.7 ± 2.0^{BCa}	3.3 ± 0.7^{BCDa}	3.2 ± 0.7^{BCDEa}
SL (2.4%)/SD (0.25%)	2.0 ± 0.9^{DEa}	2.1 ± 0.4^{CDEa}	1.9 ± 0.9^{Ca}	1.9 ± 0.2^{CDa}	2.2 ± 0.6^{DEa}
SL (2.4%)/PS (0.3%)	3.3 ± 0.3^{CDEa}	3.0 ± 0.3^{CDEa}	2.8 ± 0.4^{BCa}	2.4 ± 0.7^{BCDa}	2.9 ± 1.2^{CDEa}
SD (0.25%)/PS (0.3%)	2.8 ± 0.3^{CDEa}	2.0 ± 0.3^{CDEa}	2.5 ± 0.7^{BCa}	2.2 ± 0.6^{BCDa}	2.8 ± 0.4^{CDEa}
Nisin (500 IU/g)/SL (2.4%)	1.8 ± 0.7^{Ea}	1.7 ± 0.1^{DEa}	2.0 ± 0.3^{Ca}	1.4 ± 0.6^{Da}	1.3 ± 0.3^{Ea}
Nisin (500 IU/g)/SD (0.25%)	2.0 ± 0.9^{DEa}	1.5 ± 0.3^{Ea}	1.7 ± 0.6^{Ca}	2.2 ± 1.2^{BCDa}	1.4 ± 0.4^{Ea}
Nisin (500 IU/g)/PS (0.3%)	4.1 ± 0.6^{BCa}	2.0 ± 0.3^{CDEb}	3.5 ± 0.7^{BCab}	3.9 ± 0.1^{BCa}	3.3 ± 1.1^{BCDEab}
Guardian NR 100 (500 ppm)	3.8 ± 0.8^{BCDa}	3.7 ± 1.5^{BCa}	4.8 ± 1.5^{ABa}	4.0 ± 1.7^{Ba}	4.6 ± 0.9^{ABCDa}
Novagard CB1 (0.25%)	3.1 ± 0.5^{CDEa}	3.5 ± 0.7^{BCDa}	3.5 ± 0.2^{BCa}	3.2 ± 0.6^{BCDa}	3.8 ± 0.8^{BCDa}
Control	6.5 ± 0.1^{Aa}	6.5 ± 0.1^{Aa}	6.5 ± 0.1^{Aa}	6.5 ± 0.1^{Aa}	6.5 ± 0.1^{Aa}

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

Table 4
Effect of antimicrobial alginate coatings on the growth of *Listeria monocytogenes* on poached turkey stored at 4°C. The initial inoculation level was 3.4 log CFU/g. Data are the means of three replicates ± one standard deviation (log CFU/g).

	Weeks of Storage							
Treatments	1	2	3	4	5	6	7	8
Control	6.0±0.6^{A}	7.1±0.4^{A}	6.9±0.0^{A}	7.3±1.0^{A}	8.4±0.1^{A}	9.0±0.2^{A}	9.5±0.3^{A}	9.9±0.2^{A}
Alginate only	4.7±0.4^{A}	5.6±1.0^{A}	6.2±0.6^{AB}	6.5±0.6^{AB}	7.8±0.5^{A}	8.7±0.1^{A}	8.9±0.2^{AB}	9.6±0.1^{A}
SL (2.4%)/SD (0.25%)	3.8±1.0^{A}	2.4±0.2^{BC}	3.5±1.6^{B}	5.0±0.8^{BC}	4.5±1.9^{A}	5.5±2.2^{ABC}	5.3±1.1^{CDE}	6.4±0.3^{BC}
SL (2.4%)/PS (0.3%)	3.7±0.8^{A}	3.2±0.8^{BC}	3.7±1.1^{AB}	4.7±0.9^{BC}	4.8±1.6^{A}	4.5±1.5^{C}	4.9±1.5^{DE}	4.3±0.9^{C}
SD (0.25%)/PS (0.3%)	3.8±1.0^{A}	3.6±0.3^{BC}	3.8±1.7^{AB}	4.7±0.6^{BC}	4.4±2.2^{A}	5.5±0.8^{BC}	4.5±1.0^{E}	5.5±1.7^{C}
Nisin (500 IU/g)/SL (2.4%)	3.3±1.4^{A}	2.3±0.6^{BC}	4.2±1.0^{AB}	4.1±0.9^{C}	4.3±2.4^{A}	5.1±0.9^{BC}	5.7±2.3^{BCDE}	5.2±1.7^{C}
Nisin (500 IU/g)/SD (0.25%)	3.5±1.5^{A}	2.0±0.6^{C}	3.7±1.4^{AB}	4.8±1.2^{BC}	4.4±2.0^{A}	6.0±1.0^{ABC}	4.6±0.8^{E}	5.0±1.4^{C}
Nisin (500 IU/g)/PS (0.3%)	3.4±1.5^{A}	2.5±0.7^{BC}	3.5±1.4^{B}	4.5±0.7^{BC}	4.4±2.1^{A}	5.4±0.7^{BC}	6.1±0.9^{ABCDE}	5.7±0.8^{BC}
Guardian NR 100 (500 ppm)	4.4±1.1^{A}	3.7±0.8^{B}	4.7±1.0^{AB}	6.3±0.2^{ABC}	6.8±0.8^{A}	8.0±0.7^{AB}	8.6±1.5^{ABC}	8.5±0.6^{AB}
Novagard CB1 (0.25%)	4.9±1.0^{A}	3.6±0.2^{BC}	5.0±0.3^{AB}	6.2±0.6^{ABC}	7.1±0.3^{A}	7.9±0.8^{AB}	8.2±1.1^{ABCD}	8.5±0.7^{AB}

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

Table 5

Effect of antimicrobial alginate coatings on the growth of aerobic and anaerobic bacteria in poached turkey stored at 4°C. The initial aerobic population for the aerobes was 2.2 log CFU/g and 1.4 log CFU/g for anaerobes. Data are the means of three replicates ± one standard deviation (log CFU/g).

	2 weeks		4 weeks		6 weeks		8 weeks	
Treatments	Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes
Control	5.2 ± 1.5^{A}	4.9 ± 0.9^{A}	6.2 ± 1.1^{A}	5.8 ± 1.2^{A}	7.8 ± 0.5^{A}	7.2 ± 0.9^{A}	8.4 ± 1.0^{A}	9.1 ± 0.4^{A}
Alginate only	4.0 ± 1.2^{AB}	4.7 ± 1.7^{AB}	5.7 ± 0.8^{A}	5.5 ± 1.0^{AB}	7.5 ± 0.6^{AB}	7.0 ± 0.9^{A}	8.4 ± 0.4^{A}	8.4 ± 0.4^{AB}
SL (2.4%)/SD (0.25%)	1.7 ± 0.1^{BC}	1.9 ± 0.3^{C}	3.3 ± 1.3^{A}	3.4 ± 1.1^{B}	5.8 ± 0.5^{BC}	5.7 ± 1.8^{A}	7.3 ± 1.0^{A}	7.7 ± 0.2^{B}
SL (2.4%)/PS (0.3%)	1.5 ± 0.1^{BC}	1.7 ± 0.6^{C}	3.5 ± 1.1^{A}	3.4 ± 0.5^{B}	5.7 ± 0.5^{BC}	5.4 ± 1.5^{A}	6.5 ± 1.0^{A}	7.6 ± 0.2^{B}
SD (0.25%)/PS (0.3%)	1.7 ± 0.3^{BC}	1.4 ± 0.4^{C}	3.8 ± 1.1^{A}	3.8 ± 0.6^{AB}	5.4 ± 0.8^{C}	5.9 ± 1.1^{A}	7.0 ± 0.4^{A}	7.4 ± 0.3^{B}
Nisin (500 IU/g)/SL (2.4%)	1.5 ± 0.2^{C}	1.7 ± 0.4^{C}	3.4 ± 1.5^{A}	3.5 ± 0.7^{B}	5.4 ± 0.8^{C}	5.3 ± 1.4^{A}	6.3 ± 1.2^{A}	7.4 ± 0.3^{B}
Nisin (500 IU/g)/SD (0.25%)	1.8 ± 0.2^{BC}	1.7 ± 0.1^{C}	3.2 ± 1.4^{A}	3.4 ± 0.3^{B}	5.1 ± 0.5^{C}	5.2 ± 1.2^{A}	6.2 ± 1.0^{A}	7.5 ± 0.0^{B}
Nisin (500 IU/g)/PS (0.3%)	$1.5\ \pm0.1^{C}$	1.8 ± 0.6^{C}	3.7 ± 1.7^{A}	3.7 ± 0.2^{AB}	5.6 ± 0.8^{BC}	5.9 ± 1.5^{A}	6.4 ± 0.9^{A}	7.7 ± 0.6^{B}
Guardian NR 100 (500 ppm)	3.1 ± 1.6^{ABC}	2.1 ± 1.2^{C}	5.2 ± 0.8^{A}	4.9 ± 1.0^{AB}	5.7 ± 0.6^{BC}	6.0 ± 1.4^{A}	7.6 ± 0.4^{A}	8.1 ± 0.6^{AB}
Novagard CB1 (0.25%)	2.1 ± 1.2^{BC}	2.4 ± 1.0^{BC}	4.7 ± 0.6^{A}	4.4 ± 0.6^{AB}	5.6 ± 1.0^{BC}	6.0 ± 1.2^{A}	7.6 ± 0.7^{A}	8.1 ± 0.5^{AB}

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

Table 6
Effect of antimicrobial alginate coatings on the growth of *Listeria monocytogenes* on roasted turkey stored at 4°C. The initial inoculation level was 3.0 log CFU/g. Data are the means of three replicates ± one standard deviation (log CFU/g).

	Weeks of Storage							
Treatments	1	2	3	4	5	6	7	8
Control	5.6±0.8^{A}	6.3±1.0^{A}	6.2±0.8^{A}	6.5±1.2^{A}	6.8±1.9^{A}	7.4±0.8^{A}	8.2±0.7^{A}	7.9±1.6^{A}
Alginate only	4.8±0.4^{A}	5.9±1.3^{A}	5.2±0.7^{AB}	6.4±1.4^{A}	6.8±1.3^{A}	7.1±0.6^{A}	8.1±0.7^{A}	7.8±0.8^{A}
SL (2.4%)/SD (0.25%)	3.2±0.6^{A}	4.6±1.6^{A}	4.7±0.5^{AB}	5.9±0.6^{A}	6.0±2.1^{A}	6.0±2.4^{A}	6.2±1.8^{A}	7.1±0.8^{A}
SL (2.4%)/PS (0.3%)	3.4±0.8^{A}	4.4±1.6^{A}	4.2±0.6^{B}	5.5±1.2^{A}	6.1±1.4^{A}	4.9±2.1^{A}	5.9±1.4^{A}	6.5±1.0^{A}
SD (0.25%)/PS (0.3%)	3.2±0.6^{A}	4.6±1.2^{A}	4.3±0.6^{AB}	5.4±1.6^{A}	4.6±2.2^{A}	5.0±2.3^{A}	6.2±1.9^{A}	6.6±1.1^{A}
Nisin (500 IU/g)/SL (2.4%)	2.7±1.4^{A}	4.1±0.0^{A}	5.1±0.8^{AB}	5.7±0.5^{A}	6.0±0.9^{A}	6.3±1.1^{A}	6.4±1.8^{A}	6.8±1.2^{A}
Nisin (500 IU/g)/SD (0.25%)	3.2±1.2^{A}	4.6±2.1^{A}	5.4±0.2^{AB}	5.6±1.4^{A}	6.1±1.7^{A}	5.9±1.5^{A}	6.4±1.9^{A}	7.3±0.7^{A}
Nisin (500 IU/g)/PS (0.3%)	3.2±1.2^{A}	4.6±1.0^{A}	4.9±1.1^{AB}	5.9±0.9^{A}	5.2±2.8^{A}	5.8±1.9^{A}	6.0±1.9^{A}	7.0±0.6^{A}
Guardian NR 100 (500 ppm)	4.6±1.2^{A}	5.4±1.0^{A}	5.9±0.3^{AB}	6.4±0.7^{A}	6.3±1.7^{A}	6.5±1.2^{A}	6.4±1.9^{A}	7.2±1.3^{A}
Novagard CB1 (0.25%)	4.4±1.5^{A}	5.1±0.9^{A}	5.4±0.6^{AB}	6.1±0.9^{A}	5.8±1.7^{A}	6.1±1.1^{A}	6.3±1.8^{A}	7.0±1.6^{A}

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

Table 7
Effect of antimicrobial alginate coatings on the growth of aerobic and aerobic bacteria in roasted turkey stored at 4°C. The initial aerobic population was of aerobes was 1.6 log CFU/g and 1.3 log CFU/g for anaerobes. Data are the means of three replicates ± one standard deviation (log CFU/g).

	2 weeks		4 weeks		6 weeks		8 weeks	
Treatments	Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes	Aerobes	Anaerobes
Control	6.9±0.7^{A}	6.5±0.2^{A}	7.3±0.3^{A}	6.5±0.8^{A}	6.9±0.7^{A}	6.1±1.1^{A}	7.4±0.5^{A}	7.5±1.2^{A}
Alginate only	6.3±1.2^{A}	6.0±1.2^{A}	7.1±0.5^{A}	6.4±0.8^{A}	6.6±0.6^{A}	6.0±1.0^{A}	6.9±0.5^{A}	7.1±1.3^{A}
SL (2.4%)/SD (0.25%)	4.5±1.0^{A}	4.8±0.3^{A}	6.6±0.1^{A}	6.4±1.1^{A}	6.0±1.0^{A}	5.3±0.3^{A}	6.7±0.9^{A}	6.9±1.3^{A}
SL (2.4%)/PS (0.3%)	5.1±1.5^{A}	5.4±1.1^{A}	6.5±0.2^{A}	5.7±0.9^{A}	6.2±0.7^{A}	5.5±0.4^{A}	5.7±0.4^{A}	6.8±0.9^{A}
SD (0.25%)/PS (0.3%)	4.4±1.0^{A}	4.7±0.1^{A}	6.6±0.2^{A}	6.0±1.0^{A}	5.7±0.9^{A}	5.7±0.5^{A}	6.4±0.7^{A}	6.4±0.8^{A}
Nisin (500 IU/g)/SL (2.4%)	5.1±0.4^{A}	4.8±0.3^{A}	6.3±1.0^{A}	6.3±0.9^{A}	6.6±0.9^{A}	5.9±0.9^{A}	6.8±0.7^{A}	6.9±1.5^{A}
Nisin (500 IU/g)/SD (0.25%)	5.2±0.6^{A}	5.5±1.0^{A}	6.3±0.9^{A}	6.2±0.6^{A}	6.3±0.8^{A}	5.6±0.7^{A}	6.7±0.5^{A}	7.1±1.1^{A}
Nisin (500 IU/g)/PS (0.3%)	4.6±0.5^{A}	4.3±0.6^{A}	5.8±1.0^{A}	5.6±1.3^{A}	6.3±0.6^{A}	5.8±1.0^{A}	6.6±0.7^{A}	6.7±1.0^{A}
Guardian NR 100 (500 ppm)	6.3±0.7^{A}	5.4±0.7^{A}	6.4±0.7^{A}	6.0±0.7^{A}	6.7±0.9^{A}	5.9±1.1^{A}	7.1±0.5^{A}	7.2±1.3^{A}
Novagard CB1 (0.25%)	5.8±0.2^{A}	5.4±1.1^{A}	6.3±1.0^{A}	5.7±1.1^{A}	6.6±0.7^{A}	5.7±1.5^{A}	6.1±1.0^{A}	7.0±1.0^{A}

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

Chapter 4

INFLUENCE OF PRIOR GROWTH CONDITIONS, PRESSURE TREATMENT PARAMETERS AND RECOVERY CONDITIONS ON THE INACTIVATION AND RECOVERY OF *LISTERIA MONOCYTOGENES, ESCHERICHIA COLI,* AND *SALMONELLA* TYPHIMURIUM IN TURKEY MEAT

Abstract

The relatively high prevalence of *Listeria monocytogenes, Escherichia coli* O157:H7, and *Salmonella enteric* serovar Typhimurium in various food products is of great concern to the food industry. The objective of this study was to determine the pressure-inactivation of the pathogens in a representative food model as affected by growth temperature and age of the culture before pressure treatment and pressure treatment temperature. The effect of recovery or incubation temperature and gas atmosphere after pressure treatment on their recovery was also determined. The pathogens being studied were inoculated into sterile turkey breast meat to a final level of ca. 3 log CFU/g and then grown to two stages, the early stage (representative of exponential phase) and late stage (representative of stationary phase), at 15, 25, 35, and 40^{o}C. Turkey meat samples were pressure-treated at 400 and 600 MPa for 2 min at initial sample temperatures of 4, 20 and 40 ^{o}C. Following treatment, samples were microbiologically analyzed by plating on tryptic soy agar plus 0.6% yeast extract (TSAYE) and incubating the plates aerobically or by plating on TSAYE plus cysteine and incubating them anaerobically at 15, 25, 35, and 40^{o}C. Plates incubated at 15^{o}C and 25-40^{o}C were enumerated after 15 and 5 days, respectively. Pressure

inactivation of the bacterial pathogens increased as a function of the pressure levels and treatment temperatures. Generally speaking, early stage cells were more resistant than late stage cells ($P < 0.05$). The incubation gas atmosphere did not affect bacterial recovery as there were no significant differences in bacterial counts from aerobic and anaerobic atmospheres. Bacteria grown at 15-35°C underwent higher population reductions than those grown at 40°C. With regard to recovery temperatures, low temperatures promoted greater recovery of injured early and late stage cells than higher temperatures ($P < 0.05$). This study indicates the importance of the history of a bacterial culture prior to pressure treatment and optimum recovery conditions after treatment when considering the adequacy of pressure treatments to enhance the microbiological safety of foods.

Introduction

Listeria monocytogenes, Escherichia coli O157:H7 and *Salmonella enteric* serovar Typhimurium are all serious safety concern for the food industry, especially with respect to ready-to-eat (RTE) and fresh meat products. *L. monocytogenes,* a gram-positive, non-spore-forming rod-shaped bacterium, is responsible for the foodborne illness listeriosis. It is estimated that in the United States, there are annually 2500 cases of illness and 500 deaths due to the illness (Lungu and Johnson, 2005). The symptoms of listeriosis are variable and range from a mild-flu like illness to more serious complications such as meningitis, septicemia, stillbirths and abortions (Salamina et al., 1996). In general, the major symptoms of disease are limited to pregnant women, neonates, the elderly and people with weak immune systems (McLauchlin, 1997). *E. coli* O157:H7 is a gram-negative rod-shaped bacterium. It is estimated that there are approximately 63,153 annual domestically acquired episodes of *E. coli* O157:H7 (Scallen et al., 2011). The symptoms of illness due to *E. coli* O157:H7 bloody diarrhea and abdominal cramps with little or

no fever. The infection can also progress to hemolytic uremic syndrome, hemolysis, thrombocytopenia, renal failure, and occasionally death (Vogt and Dippold, 2002). *S.* Typhimurium is a gram-negative rod-shaped bacterium. It is estimated that there are approximately 1 million annual domestically acquired episodes of nontyphodial *Salmonella* (Scallen et al., 2011). *S.* Typhimurium can often cause the food-borne illness, salmonellosis. Common symptoms associated with salmonellosis can range from diarrhea to typhoid fever. Also with the ingestion of the pathogen serious health issues can arise because once the bacterium is ingested it can disseminate into the liver or spleen via the bloodstream (McGhie et., al 2009).

A variety of food products have been implicated in outbreaks of *L. monocytogenes, S.* Typhimurium, and *E. coli* including meats, produce, and dairy. RTE meat and poultry products are fully processed and should be free of pathogens (Huang 2004). However, various instances of foodborne disease outbreaks have been reported in recent years, linked with pathogens, notably *L. monocytogenes* (CDC, 1998; CDC, 2000; CDC, 2002). Although thermal processes are used in the food industry with the goal to eliminate *L. monocytogenes* from RTE meat and poultry products, cross-contamination can occur. During a joint study conducted at a turkey frankfurter production site, researchers from the U.S. Centers for Disease Control and Prevention and the U.S. Department of Agriculture identified that the operation of peeling immediately following thermal processing was the main critical stage where recontamination of franks by *L. monocytogenes* took place before final packaging (Wenger et al., 1990). Hence, RTE poultry products contaminated with this potentially deadly pathogen could enter the market, causing food poisoning especially among the high risk groups of the population. Contamination of foods

processed by low temperature cooking by *Salmonella and E. coli* O157:H7 have also been reported (Huang, 2004).

The effectiveness of high pressure processing on inactivating microorganisms can be affected by a variety of factors. Shearer et al. (2010) studied the effects of growth and recovery temperatures on pressure resistance of early stationary phase *L. monocytogenes* in milk. It was found that there was no significant difference in pressure resistance of *L. monocytogenes* grown at 10 to 25°C with approximately 6.5-log CFU/ml population reductions. At growth temperatures greater than 25 °C, pressure resistance increased with less than 1-log CFU/ml reduction observed for *L. monocytogenes* originally grown at 43°C. After pressure treatment, regardless of growth temperature and pressure treatment, the greatest recovery of *L. monocytogenes* was within the 4 to 20°C range. McClement et al. (2001) found that exponential-phase cells were less resistant to pressure than stationary-phase cells for *L. monocytogenes*, *Bacillus cereus*, and *Pseudomonas fluorescens*. In addition, growth temperature was found to have a significant effect at the two growth stages studied. Exponential cells grown at 8°C were more resistant than those grown at 30°C, but for stationary-phase cells the reverse was true. Aertsen et al. (2005) reported that *E. coli* survivor counts were considerably higher on plates incubated under anaerobic conditions than those incubated aerobically. These results indicate that appropriate storage or incubation conditions after pressure treatment, including gas atmosphere, may be critical to the recovery of pressure-injured cells. Thus, determination of adequate pressure processing parameters and characterization of bacterial growth parameters such as culture and recovery temperatures and gaseous atmospheres for maximal recovery are needed to ensure that optimum processing conditions are selected.

The objectives of this study were to: (i) determine the effect of prior growth temperature and growth phase on the pressure resistance of *L. monocytogenes*, *S.* Typhimurium and *E. coli* O157:H7 prior to pressure treatment, (ii) determine the effect of pressure treatment temperature on the inactivation of the pathogens, and (iii) determine the effect of recovery or incubation temperature and gas atmosphere after pressure treatment on the recovery of the pathogens.

Materials and Methods

2.1 Bacterial strains and culture conditions

L. monocytogenes ATCC 19115 (serotype 4B), *S.* Typhimurium DT104, and *E. coli* O157:H7 (strain 1730) strains were used. Surveys of serotype distribution by Ero and Ayaz (2011) of *L. monocytogenes* isolated from turkey meat previously revealed that serotype 4B was among one of the most predominate serotypes (> 95%) isolated from contaminated food samples and in cases of human listeriosis. The *S.* Typhimurium DT 104 strain was isolated from poultry while *E. coli* O157:H7 was an outbreak isolate. The strains were maintained on tryptic soy agar plus 0.6% yeast extract (TSAYE) (Difco Laboratories, Sparks, MD) plates and stored at 4°C. Each strain was grown separately in tryptic soy broth with 0.6% yeast extract (TSBYE) for 24 h at 35°C and 100 µl of each overnight culture was transferred to fresh TSBYE broths for 24-h incubation. On the day of the experiment, each strain was readjusted with 0.1% peptone water to cell densities of ca. 10^5 CFU/ml, which served as the inoculum. Serial dilutions were plated onto TSAYE plates and incubated at 35°C for 24 h to determine initial cell numbers.

2.2 Determination of growth curves

Raw sliced turkey was obtained from a local grocery store, kept frozen at -20°C and thawed at 4 ± 1°C for 1 day immediately before use. The raw turkey was cut into 20 ± 1 g pieces; each piece was placed in a separate beaker and covered with aluminum foil. The turkey samples

were autoclaved for 45 min and then stored at 4 ± 1°C. The turkey had a pH of 6.1 and a water activity of 0.929. The turkey samples were inoculated with a 95-μl aliquot of a 10^5 CFU/ml dilution of *L. monocytogenes, S.* Typhimurium, or *E. coli* O157:H7 to achieve a final concentration of ~10^3 CFU/g. Each sample was placed at its respective growth temperatures of 15, 25, 35, and 40°C. At selected time intervals, microbial counts in the samples were determined. The times of analysis were 0 (initial inoculation level), 48, 72, 96, 120, and 144 h for the 15°C growth temperature, 0, 24, 32, 40, 48, and 56 h for the 25°C growth temperature, 0, 14, 18, 22, 26, and 30 h for the 35°C growth temperature, and 0, 8, 12, 16, 20, and 24 h for the 40°C growth temperature.

2.3 Microbial enumeration of inoculated samples

After the specified time of storage for each growth temperature, the turkey samples were individually placed in larger stomacher bags containing approximately 180 ml of 0.1% sterile peptone water and stomached for 2 min. The amount of peptone water added to the turkey meat in the stomacher bag was relative to the weight of the turkey meat; the ratio of sample to peptone water was 1:9. Serial dilutions were made in 0.1% peptone water, and counts of *L. monocytogenes, S.* Typhimurium, and, *E. coli* O157:H7 were determined by plating the serial dilutions onto TSAYE plates. The plates were incubated at 35°C for 48 h and then the plates were counted.

2.4. Determining the effect of growth temperature on the pressure resistance of L. monocytogenes, S. Typhimurium, and E. coli O157:H7 and the growth conditions after pressure treatment on their recovery

The turkey was prepared in the same way as prepared for the growth curve determination; the only difference being that the turkey was cut into 2 g pieces instead of 20 g pieces. Each of the turkey samples was inoculated with a 9.5 μl aliquot of a 10^5 CFU/ml dilution of *L. monocytogenes, S.* Typhimurium, or *E. coli* O157:H7 to achieve a final concentration of ~10^3 CFU/g. Each sample was placed at its respective growth temperatures of 15, 25, 35, and 40°C until the sample had reached the determined early stage or late stage times. The inoculated samples were then double sealed in two pouches and treated in an Avure PT-1 laboratory-scale pressure unit (Avure Technologies Inc., Kent, WA, USA) monitored with DASYLab ® 7.0 software (DASYTEC USA, Bedford, NH). The samples were treated at 400 and 600 MPa at initial sample temperatures of 4, 20, and 40^{o}C for 2 min. High pressure treated samples were microbiologically analyzed for *L. monocytogenes, S.* Typhimurium, or *E. coli* O157:H7 by following the procedure above except the samples were plated onto two types of plates, TSAYE and TSAYE supplemented with cysteine (0.1%) (TSAYE-C) (Suh and Knabel, 2001). The supplementation of cysteine was to enhance the recovery of pressure-injured cells under anaerobic condition. The TSAYE plates were incubated aerobically while the TSAYE-C plates were incubated anaerobically in plastic bags made of Mylar laminated with aluminum (Sorbent Systems, Los Angeles, CA) with GasPak (BD BBL, BD, Franklin Lakes, NJ). A preliminary study indicated that these bags performed as well as anaerobic jars (BD BBL GasPak Jar Systems). Both types of plates, aerobic and anaerobic, were incubated at 15, 25, 35, and 40°C. The plates at 15°C were counted after 15 days of incubation and the plates at 25°C, 35°C, and 40°C were counted after 5 days of incubation.

2.5 Statistical analysis

Three independent trials were conducted for all the experiments. Where appropriate, statistical analyses were conducted using JMP® 8.0.1 (SAS Institute Inc.,Cary, NC). One-way analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were used to determine differences in the populations of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7. The following parameters were compared, growth phase, growth temperature, pressure treatment temperature, recovery temperature, and gas atmosphere. Significant differences were considered at the 95% confidence level ($P < 0.05$).

Results

3.1 Determination of growth curves

The growth curves are shown in Figure 1. The growth curves were determined to establish the times at which early (representative of exponential phase) and late (representative of stationary phase) stages were achieved. The times to reach early (ca. 10^7 CFU/g except for *L. monocytogenes* at 40°C which was ca. 10^5 CFU/g) and late stages (ca. 10^9 CFU/g except for *L. monocytogenes* at 40°C which was ca. 10^6 CFU/g) were shown in Table 1. These incubation times were used for all subsequent experiments.

3.2 Effect of the growth temperature before pressure treatment on pressure resistance of the pathogens

The growth temperature before pressure treatment had an effect on the effectiveness of the treatment; cells grown at 40°C in most of the cases were more pressure resistant than those grown at 15, 25, and 35°C (Table 2). For example, when the stationary phase cells of *E. coli* O157:H7 were treated at 400 MPa/20°C and incubated at 35°C aerobically, the log reductions were 2.8, 2.4, 2.3 and 1.6 for growth temperatures of 15, 25, 35 and 40°C (before HHP), respectively.

3.3 Effect of the physiological age on pressure resistance of the pathogens

The early stage populations were more resistant to the pressure treatments than the late stage populations (Table 2). This trend can be observed by comparing the population of *L. monocytogenes* grown at 15^{o}C, treated at 400 MPa and 20^{o}C, and incubated at 35^{o}C aerobically. The log reductions were 5.2 and 3.7 for the late stage and early stage cells, respectively.

3.4 Effect of pressure magnitude on pressure inactivation of the pathogens

Increasing the pressure level from 400 to 600 MPa increased the lethal effect of HHP at all treatment temperatures for both exponential and stationary phase cells regardless of their growth temperatures ($P < 0.05$) (Table 2). For instance, a treatment of 400 MPa for 2 min at 4^{o}C reduced the counts of *L. monocytogenes* grown at 15°C to log phase by 3.8 when the treated samples were incubated aerobically at 25^{o}C, while the higher pressure level at 600 MPa reduced the counts by 5.1 log when other conditions remained the same. These differences in inactivation were also more pronounced at 15^{o}C growth and recovery temperatures. In addition, *S.* Typhimurium DT 104 and *E. coli* O157:H7 were generally more pressure resistant than *L. monocytogenes*.

3.5 Effect of treatment temperatures on pressure inactivation of the pathogens

The inactivation ratios were directly related to treatment temperatures and ranked in the order of 40°C > 20° > 4°C. For instance, when turkey meat was inoculated with *S.* Typhimurium grown to stationary phase at 15°C, treated at 400 MPa at 4, 20 and 40^{o}C and recovered aerobically at 15^{o}C after pressure treatment, the log reductions were 2.8, 3.1 and 3.6, respectively.

3.6 Effect of the growth conditions after pressure treatment on the recovery of the pathogens

There was no appreciable difference in the bacterial recovery when incubated aerobically

as opposed to anaerobic conditions (Table 2). This observation was consistent across all three organisms, growth temperatures, pressure levels and treatment temperatures. For instance, when *E. coli* cells were grown at 25^{o}C to stationary phase, pressure-treated at 400 MPa and 40^{o}C and recovered at 40^{o}C, the aerobic and anaerobic counts were 3.5 and 3.7 log CFU/g, respectively. For the 4^{o}C treatment temperature the log reductions were 2.5 and 2.4 log CFU/g, respectively with all other parameters the same.

For both the exponential and stationary phase cells, the lower the recovery temperature the better the recovery of the cells; thus the 40^{o}C recovery temperature had the highest log reductions and the 15^{o}C had the lowest. This trend can be observed through the *S.* Typhimurium grown at 15^{o}C to stationary phase, treated at 600 MPa and 4^{o}C, and incubated aerobically after pressure treatment; the log reductions were 4.2, 4.5, 5.0, and 5.4 for recovery temperatures of 15, 25, 35 and 40^{o}C, respectively. This trend can also be observed through the exponential phase with *E. coli* grown at 40^{o}C, treated at 400 MPa and 4^{o}C, and incubated aerobically after pressure treatment; the log reductions were 0.9, 1.3, 1.5, and 1.7 for recovery temperatures of 15, 25, 35 and 40^{o}C, respectively.

Discussion

Factors that influences pressure resistance of bacteria include the inherent piezoresistance of the organism, which varies between species and strains, physiological state (age of culture/growth phase) of the cells during exposure to pressure treatment, growth temperature of the microorganism prior to pressure treatment, and physicochemical characteristics of the medium in which microorganisms are suspended. The extent of microbial inactivation by pressure depends on treatment time, temperature and pressure level applied. Factors affecting microbial recovery of pressure-injured cells include incubation (storage) temperature after

pressure treatment, gas atmosphere and plating media. In this study a comprehensive experiment was conducted to determine the effect of several of these factors on the pressure resistance and inactivation of *L. monocytogenes, S.* Typhimurium, and, *E. coli* O157:H7 and on their recovery after pressure treatment.

S. Typhimurium DT 104 and *E. coli* O157:H7 were generally more pressure resistant than *L. monocytogenes*. Various authors have mentioned that Gram-positive organisms are more pressure resistant than Gram-negative cells, and it has been postulated that the cell membrane is more complex in Gram-negative bacteria and thus more susceptible to environmental changes caused by the pressure treatment (Shigehisa et al., 2001). However, our findings revealed that the order of baroresistance increased in the order of *L. monocytogenes* <*Salmonella* Typhimurium < *E. coli* O157:H7. Indeed, it has been reported that *E. coli* O157:H7 is one of the few gram- negative known to be highly pressure resistant vegetative pathogens to date (Patterson et al., 1997). This order was similar to that observed by Chen et al. (2005) who demonstrated that there was no correlation between piezotolerance and bacterial taxonomy. Despite the fact that these results were obtained using only one strain of the organism and the existence of considerable variation in the pressure sensitivity of the strains, other researchers have also reported similar trends of the same bacterial species in other food matrices. Neetoo et al. (2009, 2010) and Black et al. (2009) demonstrated the high pressure tolerance of several strains of E. coli O157:H7 including Strain 1730 used in this study on alfalfa seeds and frozen beef, respectively. Guan et al. (2005) and Neetoo et al. (2009) also previously demonstrated the high pressure-resistance of *Salmonella* DT 104 in milk and alfalfa seed, respectively. The pressure resistance of *L. monocytogenes* ATCC 19115 was also previously investigated by Shearer et al. (2010).

The data indicate that the different growth temperatures before pressure treatment had an impact on the pressure resistance of the cells; generally the 40^{o}C growth temperature had a smaller log reduction in the bacterial populations than the 15^{o}C, 25^{o}C, and 35^{o}C growth temperatures. Other studies have also found similar results regarding growth temperature; it was found that *L. monocytogenes* pressure resistance increased with an increasing growth temperature (Shearer et al., 2010). Casadei et al. (2002) showed that exponential-phase cells of *E. coli* NCTC 8164 exhibited maximum pressure resistance when grown at 10°C and decreased resistance with increasing growth temperature. In the same study stationary-phase cells exhibited lowest pressure-resistance at 10 and 45°C with a peak for maximum pressure resistance around optimal growth temperatures of 30-37°C. This dependence of pressure resistance on growth temperature can be attributed to membrane fluidity and fatty acid content. The fluidity of membrane lipid bilayers is governed by the ratio of saturated to unsaturated fatty acids in membrane phospholipids, which in turn is affected by the growth temperature (Morein et al., 1996). Cells grown at lower temperatures incorporate a higher fraction of unsaturated fatty acids in their membrane phospholipids to maintain fluidity of the membranes (Russell et al., 1995). In both exponential and stationary-phase cells, as growth temperature is increased, it is accompanied by a continuous increase in membrane fluidity (Manãs and Mackey, 2004). Thus, growth temperature dictates the dynamic fluidity state of membranes, which in turn indirectly affects their degree of pressure-sensitivity.

Another observation made in this study is that early stage cells, representative of exponential phase, were more resistant to the pressure treatments than the late stage populations, representative of stationary phase. Several authors have shown that the growth phase of bacteria plays a role in determining their pressure resistance/sensitivity. Generally it has been observed

that cells in the stationary phase of growth are more pressure resistant than those in the exponential phase; specifically *L. monocytogenes* (NCTC and Scott A), *Bacillus cereus* (NCFB 578 and 1031), *Pseudomonas fluorescens* (ANA11 and NCDO 1524), *E. coli* strain J1, and *L. monocytogenes* ATCC 19115 (McClements et al., 2001; Manãs and Mackey, 2004; Hayman et al., 2007). However those studies showing that stationary phase cells are more pressure resistant then exponential phase cells followed a similar methodology; those studies grew the bacteria to the desired phase in nutrient media then added the already exponential or stationary phase cells to the studied food right before the pressure treatment. In our experiment the three pathogens were grown to the desired phase in the food (turkey in this case) itself. Thus it is possible that the media/matrix that the food is grown in could affect the response of the bacteria to pressure.

Throughout the data it appears that the higher the pressure treatment temperature the more effective the treatment. Other studies conducted on the pressure treatment temperature have similarly found that increasing temperature will cause loss of viability in most pathogens (Alpas et al., 2000). Temperature during HPP treatment can indeed exert a significant impact on microbial survival and subsequent growth (Hogan et al., 2005). Increased inactivation is usually observed at temperatures above ambient temperature. The combination of elevated temperatures (< 50°C) with pressure has been suggested as a practical way to overcome the problem of pressure-resistant strains of vegetative cells. Temperature and HHP can cause considerable microbial inactivation when applied alone, but it has been observed that when these two treatments are combined, they can confer dramatically improved inactivation ratios. Patterson and Kilpatrick (1998) also reported the synergistic inactivation of a pressure-resistant strain of *E. coli* O157:H7 when subjected to HHP at 400 MPa at 50°C. The authors showed that neither temperature nor HHP alone could achieve the level of inactivation reported (5 – 6 log reduction).

Another trend observed throughout the data is that there is no observable difference between the aerobic and anaerobic data. Previous studies have demonstrated that the extent of bacterial recovery after pressure treatment is dependent on the gaseous composition of the storage atmosphere. Ye et al. (2011) showed that aerobic incubation slightly promoted greater recovery of pressure-treated cells of *Vibrio parahaemolyticus* inoculated into oyster meats although there was no statistically significant difference in the counts between aerobic and anaerobic growth conditions. Work conducted by Bull et al. (2005) indicated that recovery of pressure-injured *L. monocytogenes* in milk occurred equally well or better in aerobic enrichment broth than in anaerobic enrichment broth. On the other hand, Aertsen et al. (2005) found that when *E. coli* MG1655 was pressure treated at 300 and 400 MPa then stored aerobically and anaerobically, the anaerobic conditions led to improved cellular survival. In the studies by Ye et al. (2001) and Bull et al. (2005), the bacteria were grown in nutrient media and then added to the studied food before the pressure treatment; while in the study by Aersten et al. (2005), the pressure treatment was performed on the *E. coli* cells grown in a nutrient broth. In our experiment the three pathogens were grown in the turkey meat before pressure treatment. Thus it is possible that the media/matrix that the bacteria are treated in or the media the bacteria is grown in could affect the gaseous conditions in relation to cell survival.

Finally, it can be observed that post-HHP bacterial recovery was greater at lower temperatures (15-35°C) than at the higher temperature of 40°C. Indeed, it has been shown that the lower recovery temperature promotes faster recovery (Shearer et al., 2010). Russell (2002) previously mentioned that pressurization can induce the formation of pores in membranes that may be more difficult to repair and reseal if the intermolecular forces stabilizing the membranes are weakened by warming. Hence, higher recovery temperatures may render membrane resealing

more difficult following structural changes caused by high pressure processing. Indeed, Russell (2002) mentioned that the fatty acyl composition of membranes can impact bacterial recovery. An increase in the extent of fatty acyl unsaturation, cis-trans unsaturation ratio or shortening of the average acyl chain length may all lower the transition temperature from a liquid crystalline to a gel phase and hence preserve membrane fluidity that is necessary for bacterial survival and growth (Russell, 1989).

Conclusion

This study has led to several important conclusions or trends that can be seen throughout the data. The growth history of cells of major foodborne pathogens, *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*, influences sensitivity to pressure treatment to a major extent, with early stage cells exhibiting higher pressure tolerance than their late stage counterparts. As anticipated, we observed that the lethality of the process was directly related to processing parameters such as the pressure magnitude and treatment temperatures for all pathogens. With regard to recovery conditions, temperatures $\leq 35°C$ promoted greatest recovery regardless of the incubation gaseous composition. The findings garnered from this study indicate that various extrinsic growth, processing and storage parameters need to be carefully manipulated to properly assess the adequacy of high pressure treatments in targeting the major foodborne pathogens in foods.

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Figures

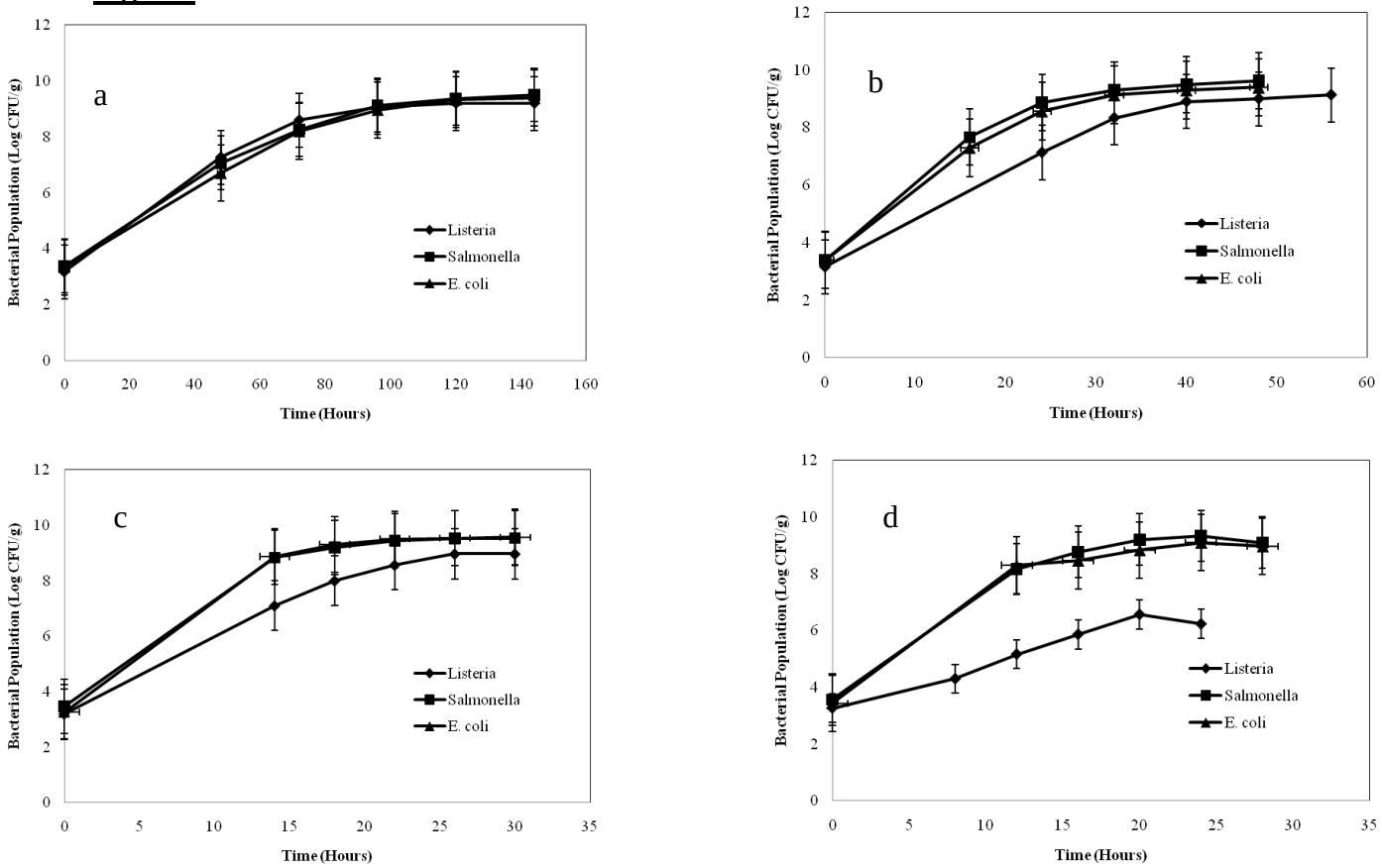

Figure 1
Growth curves of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium in turkey breast meat at different growth temperatures, (a) 15^{o}C, (b) 25^{o}C, (c) 35^{o}C and (d) 40^{o}C.

Tables

Table 8

The incubation times required to reach early stage and late stage at growth temperatures of 15, 25, 35 and 40°C

Growth	Time to early stage (h)			Time to late stage (h)		
Temp.	*L. monocytogenes*	*E. coli*	*Salmonella*	*L. monocytogenes*	*E. coli*	*Salmonella*
15°C	48	48	48	120	144	144
25°C	24	16	13	48	48	48
35°C	14	9	9	30	26	26
40°C	12	8	9	24	24	24

Table 9

Effect of growth temperature, growth phase, pressure treatment temperature and growth conditions after HHP on the inactivation of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* in turkey breast meat and their recovery. The pathogens were grown in sterile turkey breast to early and late stages at 15, 25, 35 and 40°C and treated at 400 and 600 MPa for 2 min at 4, 20, and 40°C. Treated samples were plated and plates were incubated at 15, 25, 35 and 40°C aerobically and anaerobically. The data are represented as Log CFU/g reduction (=log #initial inoculation level – log #survivors). Data are the means of three replicates ± one standard deviation (log CFU/g).

(A) *L. monocytogenes*

(1) 15°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	4.6±0.1Aa	4.6±0.2Aa	4.2±0.4Aa	1.3±0.3Ab	6.3±0.2Aa	6.1±0.1Aa	5.7±0.2Aa	3.0±0.4Ab
20°C	5.0±0.2Ba	4.9±0.1Aa	4.6±0.2ABa	1.9±0.3ABb	6.2±0.1Aa	6.1±0.1Aa	5.8±0.3Ba	3.0±0.2Ab
40°C	5.6±0.0Ca	5.6±0.2Ba	5.1±0.1Ba	2.7±0.5Bb	7.1±0.1Ba	7.1±0.0Ba	6.6±0.1Bb	3.9±0.2Bc
Recovery Cond.: anaerobic. Stage: Late.								
4°C	4.7±0.2Aa	4.7±0.3Aa	4.3±0.2Aa	1.3±0.1Ab	6.5±0.4Aa	6.5±0.1Aa	6.0±0.2Ab	2.9±0.1Ac
20°C	5.2±0.2Aa	4.7±0.4Aa	4.5±0.1Aa	1.8±0.5ABb	6.5±0.3Aa	6.7±0.3Aa	6.1±0.2ABa	3.3±0.1Bb
40°C	5.8±0.3Ba	5.7±0.4Ba	5.1±0.1Ba	2.6±0.5Bb	7.2±0.1Aa	7.1±0.1Ba	6.8±0.5Ba	3.6±0.2Cb
Recovery Cond.: aerobic. Stage: Early.								
4°C	3.8±0.1Aa	3.1±0.1Ab	2.6±0.2Ac	0.6±0.3Ad	4.9±0.0Aa	4.5±0.4Aab	4.0±0.1Ab	2.0±0.2Ac
20°C	3.6±0.2Aa	3.5±0.2Aa	2.9±0.7ABa	1.0±0.1ABb	4.8±0.3Aa	4.1±0.1ABb	4.0±0.3Ab	2.1±0.2Ac
40°C	4.4±0.2Ba	4.1±0.3Ba	3.7±0.1Ba	1.5±0.5Bb	5.3±0.2Aa	5.0±0.3Ba	4.8±0.3Ba	2.9±0.2Bb
Recovery Cond.: anaerobic. Stage: Early.								
4°C	4.0±0.2Aa	3.0±0.1Ab	2.7±0.2Ab	0.7±0.2Ac	5.2±0.2Aa	4.2±0.1Ab	4.0±0.2Ab	1.9±0.2Ac
20°C	4.0±0.2Aa	3.4±0.2ABb	2.8±0.2Ac	0.9±0.1ABd	4.8±0.3Aa	4.2±0.2Ab	4.0±0.2Ab	1.9±0.1Ac
40°C	4.6±0.2Ba	3.8±0.2Bb	3.6±0.2Bb	1.6±0.5Bc	5.4±0.1Aa	5.1±0.3Ba	4.5±0.3Ab	2.7±0.1Bc

(2) 25°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	4.6±0.2Aa	4.5±0.1Aa	4.3±0.3Aa	1.2±0.2Ab	6.1±0.2Aa	6.2±0.1Aa	6.0±0.3Aa	3.0±0.2Ab
20°C	5.0±0.2Aa	5.2±0.2Ba	4.7±0.3Aa	1.5±0.6Ab	6.4±0.3Aa	6.2±0.1Aa	6.1±0.3Aa	3.1±0.3Ab
40°C	5.9±0.3Ba	5.9±0.2Ca	5.3±0.2Ba	2.4±0.5Ab	7.2±0.2Ba	7.1±0.2Ba	6.9±0.3Ba	4.0±0.3Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	4.7±0.2Aa	4.7±0.2Aa	4.5±0.3Aa	1.3±0.1Ab	6.1±0.1Aa	6.4±0.4Aab	6.1±0.2Ab	2.9±0.1Ac
20°C	5.0±0.1Aa	5.0±0.4Aa	4.7±0.3Aa	1.5±0.2Ab	6.3±0.1Aa	6.5±0.4ABa	6.2±0.2Aa	3.2±0.2Ab
40°C	5.6±0.2Ba	6.0±0.3Ba	5.6±0.3Ba	2.5±0.3Bb	7.2±0.3Ba	7.2±0.2Ba	6.9±0.1Ba	3.9±0.0Bb
Recovery Cond.: aerobic. Stage: Early.								
4°C	3.8±0.1Aa	3.3±0.1Ab	3.1±0.2Ab	0.5±0.2Ac	5.1±0.1Aa	4.3±0.1Ab	4.1±0.3Ab	2.0±0.2Ac
20°C	3.7±0.3Aa	3.4±0.3ABab	2.9±0.2ABb	0.7±0.2Ac	5.4±0.3ABa	4.3±0.1Ab	4.0±0.3Ab	2.1±0.2Ac
40°C	4.8±0.1Ba	3.9±0.3Bb	3.5±0.2Bb	1.7±0.3Bc	5.6±0.1Ba	4.9±0.3Bb	4.7±0.1Bb	2.8±0.2Bc
Recovery Cond.: anaerobic. Stage: Early.								
4°C	3.9±0.0Aa	3.1±0.1Ab	2.9±0.2Ab	0.9±0.1Ac	5.1±0.3Aa	4.2±0.4Ab	4.1±0.2Ab	1.9±0.0Ac
20°C	4.0±0.4Aa	3.6±0.2ABab	3.1±0.2Ab	1.0±0.2Ac	5.4±0.1ABa	4.2±0.2Ab	3.9±0.2Ab	2.0±0.2Ac
40°C	4.6±0.2Ba	4.1±0.4Bab	3.7±0.2Bb	1.6±0.2Bc	5.8±0.2Ba	5.2±0.1Bb	4.8±0.3Bb	2.9±0.2Bc

(3) 35°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	5.0±0.1Aa	5.1±0.1Aa	4.4±0.2Ab	1.6±0.1Ac	6.7±0.1Aa	6.6±0.2Aa	6.2±0.3Aa	3.3±0.3Ab
20°C	5.2±0.2Aa	5.3±0.2Aa	4.9±0.2Aa	2.0±0.4Ab	6.6±0.1Aa	6.5±0.2ABa	6.2±0.2Aa	3.2±0.4Ab
40°C	6.2±0.2Ba	6.2±0.2Ba	5.8±0.1Ba	2.5±0.4Ab	7.6±0.3Ba	7.5±0.1Ba	7.1±0.2Ba	4.2±0.2Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	5.0±0.1Aa	5.2±0.1Aa	4.5±0.5Aa	1.6±0.5Ab	6.8±0.2Aa	6.9±0.1Aa	6.3±0.2Aa	3.1±0.5Ab
20°C	5.2±0.1Aa	5.5±0.0Aab	5.0±0.2ABb	1.9±0.3Ac	6.7±0.3Aa	6.7±0.1Aa	6.4±0.4Aa	3.1±0.5ABb
40°C	6.0±0.1Ba	6.2±0.1Ba	6.0±0.4Ba	2.4±0.6Ab	7.4±0.1Ba	7.7±0.1Ba	7.1±0.4Aa	4.1±0.3Bb
Recovery Cond.: aerobic. Stage: Early.								
4°C	3.9±0.3Aa	3.8±0.2Aa	3.5±0.2Aa	1.3±0.5Ab	5.0±0.2Aa	4.8±0.1Aa	4.7±0.3Aa	2.3±0.5Ab
20°C	3.7±0.1Aa	3.9±0.1Aa	3.7±0.2Ba	1.1±0.4ABb	5.2±0.3Aa	4.9±0.1Aa	4.7±0.2Aa	2.3±0.5Ab
40°C	4.8±0.2Ba	4.7±0.1Ba	4.2±0.1Ca	1.9±0.6Bb	5.8±0.1Ba	5.4±0.3Bab	5.2±0.2Bb	3.3±0.2Bc
Recovery Cond.: anaerobic. Stage: Early.								
4°C	3.9±0.1Aa	3.5±0.2Aa	3.5±0.3Aa	1.0±0.4Ab	5.1±0.3Aa	4.6±0.2Aa	4.5±0.2Aa	2.1±0.4Ab
20°C	3.8±0.2Aa	3.9±0.1Ba	4.0±0.3Aa	0.9±0.4Ab	5.3±0.2Aa	4.7±0.4Ba	4.8±0.3ABa	2.2±0.4Ab
40°C	4.5±0.2Ba	4.4±0.1Ca	4.4±0.3Ba	1.8±0.6Ab	5.8±0.0Ba	5.6±0.2Cab	5.2±0.3Bb	3.0±0.2Ac

(4) 40°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	5.0±0.0Aa	5.1±0.3Aa	5.0±0.2Aa	1.7±0.0Ab	6.6±0.1Aa	6.4±0.1Aa	6.5±0.4Aa	3.3±0.5Ab
20°C	5.1±0.1Aa	5.2±0.2Aa	5.2±0.2Ba	1.8±0.3Ab	6.7±0.3Aa	6.7±0.2Aa	6.4±0.3Aa	3.1±0.4Ab
40°C	5.9±0.2Ba	6.2±0.1Ba	6.1±0.4Ba	2.6±0.3Bb	7.7±0.3Ba	7.5±0.4Ba	7.5±0.1Ba	4.4±0.4Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	5.0±0.1Aa	5.2±0.2Aa	4.9±0.1Aa	1.8±0.5Ab	6.9±0.2Aa	6.4±0.3Aa	6.3±0.6Aa	3.4±0.5Ab
20°C	5.2±0.1Aa	5.2±0.2Aa	5.0±0.4Aa	1.8±0.4Ab	6.8±0.2Aa	6.6±0.2Aa	6.5±0.1Aa	3.4±0.3Ab
40°C	6.2±0.2Ba	6.0±0.2Ba	5.9±0.1Ba	3.1±0.4Bb	7.7±0.2Ba	7.7±0.1Ba	7.4±0.2Ba	4.2±0.5Ab
Recovery Cond.: aerobic. Stage: Early.								
4°C	4.2±0.1Aa	4.0±0.1Aa	4.0±0.3Aa	1.2±0.2Ab	5.4±0.1Aa	5.0±0.0Aa	4.9±0.4Aa	2.3±0.2Ab
20°C	4.4±0.2Ba	4.2±0.1Aa	4.0±0.3Aa	1.4±0.2Ab	5.2±0.1Aa	4.9±0.0Aa	5.1±0.5Aa	2.5±0.2Ab
40°C	5.0±0.1Ca	4.7±0.1Ba	4.6±0.5Aa	1.9±0.5Ab	6.0±0.1Ba	5.5±0.3Ba	5.5±0.5Aa	3.2±0.2Bb
Recovery Cond.: anaerobic. Stage: Early.								
4°C	4.6±0.1Aa	3.8±0.1Aa	3.9±0.4Aa	1.5±0.5Ab	5.2±0.3Aa	4.9±0.1Aa	4.9±0.3Aa	2.3±0.5Ab
20°C	4.4±0.2Aa	3.9±0.1Aa	4.0±0.3Aa	1.4±0.4Ab	5.3±0.3Aa	4.7±0.1Aa	5.0±0.4Aa	2.4±0.5Ab
40°C	5.0±0.1Ba	4.5±0.2Ba	4.2±0.1Aa	2.0±0.7Ab	6.1±0.1Ba	5.7±0.1Bab	5.4±0.3Ab	3.1±0.4Ac

For each recovery temperature, values in the same column followed by the same upper case letter are not significantly different ($P > 0.05$). Values in the same row followed by the same lower case letter are not significantly different ($P > 0.05$). Also statistical analysis was performed for the cluster of values within the same category of pressure level, growth phase, and recovery atmosphere.

(B) *E. coli* O157:H7

(1) 15°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	2.2±0.2Aa	2.2±0.2Aa	1.9±0.2Aab	1.5±0.1Ab	4.1±0.5Aa	3.9±0.0Aa	3.9±0.1Aa	3.5±0.1Aa
20°C	2.5±0.2Aa	2.1±0.3Aab	2.2±0.2Aab	1.7±0.3Ab	4.4±0.3ABa	4.1±0.1Aa	4.0±0.1Aa	3.2±0.1ABb
40°C	4.1±0.2Ba	3.2±0.3Bb	3.2±0.2Bb	2.6±0.1Bc	5.1±0.2Ba	4.8±0.3Ba	4.5±0.2Bab	4.0±0.3Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	2.2±0.1Aa	2.3±0.2Aab	2.0±0.2Aab	1.7±0.3Ab	4.2±0.2Aa	4.0±0.1Aa	4.0±0.3Aa	3.8±0.3Aa
20°C	2.4±0.2Aa	2.1±0.2Aab	1.9±0.1Abc	1.7±0.2Ac	4.6±0.2Aa	4.4±0.2Aa	4.1±0.2Aab	3.6±0.3Ab
40°C	4.1±0.2Ba	3.5±0.2Bb	3.1±0.2Bc	2.8±0.2Bc	5.3±0.2Ba	4.9±0.2Bab	4.5±0.3Abc	4.2±0.2Ac
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.6±0.2Aa	1.6±0.2Aa	0.9±0.4Aa	0.9±0.5Aa	2.6±0.3Aa	2.8±0.2Aa	1.7±0.4Aab	2.0±0.4Ab
20°C	1.4±0.2ABa	1.8±0.2Aa	0.9±0.4Aa	0.9±0.6Aa	2.6±0.3Aa	2.7±0.3Aa	1.9±0.5Aa	1.8±0.3Aa
40°C	2.1±0.2Ba	2.1±0.3Aa	1.6±0.4Aa	1.6±0.4Aa	3.2±0.1Ba	3.2±0.2Aa	2.3±0.2Ab	2.4±0.2Ab
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.5±0.3Aa	1.6±0.2Aa	0.9±0.3Aa	0.8±0.4Aa	2.7±0.4Aa	2.8±0.3Aa	2.2±0.3Aa	2.1±0.6Aa
20°C	1.4±0.2Aa	1.8±0.3ABab	1.1±0.3Aab	0.9±0.4Ab	2.5±0.3Aa	3.1±0.3Aa	2.4±0.4Aab	1.7±0.2Ab
40°C	2.1±0.3Ba	2.2±0.1Ba	1.6±0.4Aa	1.4±0.5Aa	3.2±0.1Aa	3.4±0.2Aab	2.8±0.3Ab	2.7±0.3Ab

(2) 25°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	1.9±0.2Aa	1.8±0.1Aa	2.0±0.2Aa	1.4±0.1Ab	3.8±0.2Aa	3.9±0.2Aa	3.7±0.2Aa	3.5±0.2Aa
20°C	1.9±0.3Aa	2.1±0.2Aab	2.2±0.2Aab	1.6±0.0Bb	4.2±0.1Ba	4.1±0.2Aa	3.9±0.2Aab	3.6±0.1ABb
40°C	3.1±0.3Ba	3.4±0.1Ba	3.2±0.0Ba	2.6±0.0Cb	5.0±0.2Ca	4.8±0.3Ba	4.8±0.3Ba	3.9±0.2Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	2.1±0.2Aa	2.1±0.1Aa	2.0±0.2Aa	1.4±0.3Ab	4.3±0.2Aa	4.1±0.2Aab	3.8±0.1Abc	3.5±0.1Ac
20°C	2.2±0.3Aa	2.2±0.1Aa	2.1±0.1Aa	1.7±0.1Ab	4.2±0.3Aa	4.2±0.2Aa	4.1±0.1Ba	3.8±0.1Aa
40°C	3.8±0.4Ba	3.7±0.3Ba	3.5±0.1Bab	2.9±0.3Bb	5.3±0.1Ba	5.2±0.3Ba	5.0±0.2Ca	4.2±0.2Bb
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.5±0.1Aa	1.7±0.2Aa	1.4±0.0Aa	1.3±0.3Aa	2.7±0.1Aa	2.9±0.5Aa	2.4±0.1Aa	2.3±0.3Aa
20°C	1.6±0.1Aa	1.8±0.2Aa	1.5±0.2Aa	1.3±0.5Aa	2.6±0.1Aa	3.0±0.5Aa	2.4±0.3Aa	2.4±0.4Aa
40°C	2.2±0.1Ba	2.4±0.3Ba	2.1±0.3Ba	1.9±0.3Aa	3.2±0.2Ba	3.4±0.4Aab	3.1±0.3Bab	2.6±0.3Ab
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.5±0.2Aa	1.8±0.2Aab	1.4±0.1Aab	1.2±0.2Ab	2.7±0.2Aa	3.0±0.4Ab	2.4±0.2Ab	2.4±0.2Ab
20°C	1.6±0.2Aa	1.9±0.3ABa	1.7±0.4ABa	1.2±0.4Aa	2.3±0.1Ba	2.9±0.4Aa	2.7±0.3Aa	2.5±0.3Aa
40°C	2.1±0.2Ba	2.6±0.3Ba	2.1±0.3Ba	2.1±0.5Ba	3.5±0.2Ca	3.5±0.4Aa	3.4±0.2Ba	2.9±0.3Aa

(3) 35°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	2.5±0.3Aa	2.2±0.2Aa	2.2±0.2Aa	1.6±0.1Ab	4.0±0.4Aa	4.0±0.3Aa	4.0±0.2Aa	3.5±0.1Aa
20°C	2.8±0.2ABa	2.4±0.2Aa	2.3±0.2Aa	1.6±0.3Ab	4.1±0.3Aa	4.3±0.2Aab	3.9±0.1Aab	3.6±0.2Ab
40°C	3.5±0.5Ba	3.5±0.2Ba	3.3±0.3Ba	3.0±0.2Ba	5.3±0.4Ba	5.1±0.2Ba	4.9±0.1Ba	4.1±0.4Ab
Recovery Cond.: anaerobic. Stage: Late.								
4°C	2.9±0.1Aa	2.2±0.2Ab	2.1±0.2Abc	1.7±0.1Ac	4.3±0.3Aa	4.2±0.2ab	4.2±0.3Aab	3.7±0.2Ab
20°C	2.7±0.1Aa	2.5±0.2Aab	2.2±0.1Ab	1.7±0.3Ac	4.5±0.1Aa	4.4±0.1a	4.0±0.2Ab	3.7±0.2Ab
40°C	4.1±0.3Ba	3.7±0.1Bab	3.4±0.4Bb	3.1±0.2Bb	5.5±0.2Ba	5.2±0.3a	5.0±0.0Ba	4.1±0.4Ab
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.2±0.4Aa	1.9±0.1Aa	1.7±0.2Aa	1.5±0.3Aa	2.6±0.3Aa	3.0±0.1Aa	2.9±0.3Aa	2.7±0.3Aa
20°C	1.5±0.3Aa	2.1±0.1Aa	1.8±0.1Aa	1.7±0.5Aa	2.5±0.4Aa	2.9±0.1Aa	2.9±0.4Aa	2.9±0.3Aa
40°C	1.9±0.3Aa	2.6±0.2Ba	2.3±0.1Ba	2.1±0.4Aa	3.2±0.2Aa	3.7±0.2Ba	3.4±0.3Aa	3.1±0.4Aa
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.4±0.3Aa	2.1±0.1Aab	1.7±0.2Ab	1.3±0.3Ab	2.9±0.3Aa	3.4±0.1Aab	2.7±0.4Aab	2.7±0.3Ab
20°C	1.7±0.4Aa	2.4±0.3ABa	1.9±0.2ABa	1.8±0.6Aa	2.5±0.2ABa	3.0±0.1Ba	2.9±0.4Aa	2.9±0.3Aa
40°C	2.1±0.4Aa	2.8±0.2Ba	2.3±0.1Ba	2.1±0.6Aa	3.5±0.3Ba	3.9±0.2Ca	3.4±0.3Aa	3.1±0.6Aa

(4) 40°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	2.7±0.2Aa	2.5±0.1Aa	2.6±0.3Aa	1.7±0.0Ab	4.3±0.3Aa	4.2±0.1Aa	4.4±0.2Aa	3.9±0.1Aa
20°C	2.9±0.4Aa	2.7±0.2Aa	2.6±0.1Aa	1.9±0.2Ab	4.6±0.2Aa	4.3±0.2Aab	4.0±0.1Bb	3.9±0.2Ab
40°C	3.8±0.4Ba	3.5±0.3Ba	3.4±0.2Ba	3.2±0.1Ba	5.3±0.2Ba	5.3±0.2Ba	5.2±0.1Ca	4.6±0.2Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	3.1±0.2Aa	2.4±0.1Aab	2.6±0.4Ab	1.8±0.1Ac	4.6±0.2Aa	4.4±0.1Aab	4.4±0.2Aab	4.1±0.1Ab
20°C	3.2±0.2Aa	2.7±0.1Ba	2.6±0.1Aa	1.8±0.4Ab	4.9±0.3Aa	4.2±0.2Ab	4.1±0.1Ab	3.9±0.1Ab
40°C	4.2±0.3Ba	3.7±0.2Cab	3.7±0.4Bab	3.3±0.1Bb	5.5±0.1Ba	5.4±0.6Ba	5.3±0.1Ba	4.8±0.1Ba
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.8±0.1Aa	1.9±0.1Aa	1.7±0.4Aa	1.7±0.1Aa	2.9±0.1Aa	3.1±0.1Aa	2.8±0.2Aa	2.7±0.2Aa
20°C	2.0±0.3Aa	2.0±0.2Aa	2.0±0.2ABa	1.8±0.2Aa	2.9±0.1Aa	3.1±0.1Aa	2.9±0.2ABa	2.7±0.3Aa
40°C	2.5±0.1Ba	2.5±0.3Aa	2.5±0.2Ba	2.4±0.2Ba	3.6±0.1Ba	3.8±0.2Bab	3.3±0.2Bb	3.3±0.2Bb
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.6±0.3Aa	1.9±0.2Aa	1.8±0.2Aa	1.9±0.1Aa	3.0±0.2Aa	3.3±0.1Aa	2.9±0.1Aa	3.0±0.1Aa
20°C	1.9±0.2ABa	2.0±0.5Aa	1.9±0.1Aa	2.2±0.3ABa	2.9±0.3Aa	3.2±0.3Aa	2.8±0.3Aa	3.0±0.2Aa
40°C	2.4±0.2Ba	2.6±0.4Aa	2.4±0.2Ba	2.7±0.3Ba	3.5±0.3Aa	3.8±0.4Aa	3.2±0.2Aa	3.7±0.2Ba

(C) *Salmonella*

(1) 15°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	2.8±0.3Aa	2.5±0.2Aab	2.1±0.1Abc	1.6±0.1Ac	4.2±0.1Aa	3.9±0.1Aa	3.5±0.1Ab	3.1±0.2Ab
20°C	3.1±0.2ABa	2.6±0.2Aab	2.2±0.3Abc	1.7±0.2Ac	4.6±0.2Ba	3.7±0.2Ab	3.5±0.3Abc	2.0±0.2Ac
40°C	3.6±0.2Ba	3.4±0.3Ba	2.9±0.0Bb	2.4±0.1Bc	5.0±0.2Ba	5.0±0.3Ba	4.0±0.3Ab	3.5±0.3Ab
Recovery Cond.: anaerobic. Stage: Late.								
4°C	2.9±0.2Aa	2.7±0.2Aa	2.1±0.1Ab	1.7±0.2Ac	4.4±0.3Aa	4.0±0.2Aab	3.4±0.1Abc	3.2±0.3Ac
20°C	3.2±0.2ABa	2.7±0.4Aab	2.1±0.2Abc	1.7±0.1Ac	4.7±0.3ABa	3.8±0.3Ab	3.5±0.1Ab	2.8±0.2ABc
40°C	3.6±0.3Ba	3.5±0.4Ba	3.1±0.2Bab	2.5±0.1Bb	5.1±0.2Ba	5.2±0.5Ba	4.0±0.1Bb	3.6±0.3Bb
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.6±0.1Aa	1.0±0.1Ab	0.6±0.3Abc	0.7±0.2Ac	2.7±0.1Aa	2.5±0.2Aab	2.1±0.2Aab	1.9±0.3Ab
20°C	1.5±0.1Aa	1.1±0.4Aa	1.0±0.3ABa	1.0±0.1Aa	2.9±0.2ABa	2.4±0.2Ab	2.4±0.3ABb	2.2±0.1Ab
40°C	2.0±0.2Ba	1.7±0.2Ba	1.6±0.3Ba	1.5±0.1Ba	3.2±0.2Ba	2.5±0.2Aab	2.6±0.1Bb	3.0±0.2Bb
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.5±0.3Aa	1.1±0.2Aab	0.6±0.1Abc	0.8±0.0Ac	2.6±0.3Aa	2.5±0.2Aa	2.3±0.1Aab	1.8±0.2Ab
20°C	1.6±0.2Aa	1.2±0.4Aa	1.0±0.2Aa	1.2±0.3Aa	2.8±0.3Aa	2.5±0.1Aab	2.4±0.2ABab	2.2±0.1Ab
40°C	2.0±0.3Aa	1.6±0.2Aa	1.7±0.2Ba	1.7±0.2Ba	3.2±0.5Aa	2.7±0.1Aa	2.7±0.2Ba	2.9±0.3Ba

(2) 25°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	2.9±0.1Aa	2.7±0.2Aa	2.3±0.2Ab	2.0±0.1Ab	4.5±0.1Aa	4.2±0.2Aab	3.9±0.2Ab	3.4±0.2Ac
20°C	2.7±0.4Aa	2.9±0.4Aab	2.1±0.2Ab	2.1±0.1Ab	4.8±0.2ABa	4.4±0.3Aa	3.7±0.1Ab	3.2±0.2ABb
40°C	4.3±0.1Ba	3.8±0.1Bb	3.3±0.2Bc	2.7±0.2Bd	5.2±0.3Ba	5.3±0.4Bab	4.6±0.2Bbc	3.9±0.2Bc
Recovery Cond.: anaerobic. Stage: Late.								
4°C	2.9±0.2Aa	2.8±0.0Aa	2.3±0.4Aab	1.9±0.3Ab	4.5±0.1Aa	4.3±0.1Aa	4.0±0.1Ab	3.3±0.1Ac
20°C	2.6±0.4Aa	2.9±0.2Aab	2.2±0.4Aab	2.0±0.2Ab	4.7±0.2ABa	4.3±0.3Aa	3.6±0.2Ab	3.2±0.2Ab
40°C	4.3±0.2Ba	3.7±0.4Bab	3.3±0.2Bb	2.6±0.1Bc	5.1±0.4Ba	5.3±0.3Bab	4.6±0.1Bbc	4.0±0.3Bc
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.1±0.1Aa	0.8±0.1Aab	0.5±0.2Ab	0.6±0.2Ab	2.6±0.2Aa	2.3±0.2Aab	2.3±0.1Aab	2.0±0.3Ab
20°C	1.2±0.2ABa	0.9±0.1Aa	0.8±0.1Aa	0.8±0.3Aa	2.8±0.1ABa	2.5±0.2ABab	2.7±0.3Aab	2.2±0.2Ab
40°C	1.5±0.1Ba	1.3±0.2Ba	1.6±0.4Ba	1.3±0.1Ba	3.2±0.2Ba	2.8±0.1Ba	2.8±0.2Aa	2.6±0.4Aa
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.2±0.2Aa	0.9±0.0Aab	0.7±0.2Abc	0.5±0.0Ac	2.6±0.1Aa	2.4±0.2Aa	2.3±0.2Aa	1.8±0.2Ab
20°C	1.2±0.4Aa	0.9±0.2ABa	0.9±0.3Aa	0.7±0.2Aa	2.9±0.2ABa	2.6±0.2Aab	2.5±0.2ABab	2.1±0.3ABb
40°C	1.4±0.1Aa	1.3±0.2Ba	1.6±0.2Ba	1.3±0.1Ba	3.2±0.2Ba	2.5±0.6Aa	2.9±0.1Ba	2.7±0.2Ba

(3) 35°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	3.2±0.1Aa	3.0±0.1Aa	2.7±0.2Ab	2.2±0.1Ac	5.0±0.1Aa	4.3±0.1Ab	3.9±0.2Abc	3.8±0.2Ac
20°C	3.4±0.1Aa	2.8±0.2Ab	2.5±0.2Abc	2.3±0.2Ac	5.3±0.2Ba	4.5±0.1Ab	4.2±0.3Ab	3.5±0.2ABc
40°C	4.0±0.1Ba	4.0±0.4Ba	3.4±0.4Bab	3.1±0.1Bb	5.7±0.0Ca	5.3±0.1Ba	5.0±0.4Ba	4.3±0.3Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	3.2±0.1Aa	3.0±0.1Aa	2.8±0.5Aa	1.9±0.2Ab	5.2±0.2Aa	4.3±0.2Ab	4.2±0.3Abc	3.6±0.2Ac
20°C	3.1±0.3Aa	2.8±0.2Aab	2.7±0.4Aab	2.1±0.3Ab	5.2±0.1ABa	4.4±0.1Ab	4.3±0.4Ab	3.7±0.4Ab
40°C	3.9±0.0Ba	4.1±0.3Bab	3.4±0.4Abc	2.9±0.2Bc	5.6±0.1Ba	5.3±0.3Ba	5.3±0.4Ba	4.4±0.5Ab
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.4±0.2Aa	1.3±0.1Aab	0.9±0.2Ab	0.5±0.1Ac	2.9±0.2Aa	2.8±0.3Aa	2.6±0.2Aab	2.1±0.3Ab
20°C	0.9±0.1Ba	1.2±0.1Aab	1.0±0.3Aab	0.6±0.2Ab	2.7±0.1Aa	2.8±0.1Aa	2.8±0.3Aa	2.3±0.3Aa
40°C	2.4±0.2Ca	2.1±0.2Bab	1.9±0.3Bb	1.3±0.1Bc	3.2±0.1Aa	3.1±0.1Aa	3.0±0.2Aa	2.8±0.3Aa
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.3±0.1Aa	1.2±0.1Aa	1.1±0.2Aab	0.7±0.3Ab	2.7±0.3Aa	2.7±0.1Aa	2.7±0.0Aa	2.3±0.4Aa
20°C	1.0±0.3Aa	1.1±0.2Aa	1.2±0.2Aa	0.7±0.5Aa	2.7±0.4Aa	2.8±0.1Aa	3.0±0.2ABa	2.5±0.2Aa
40°C	2.3±0.2Ba	2.2±0.1Ba	2.0±0.2Ba	1.3±0.2Ab	3.2±0.1Aa	3.3±0.1Ba	3.2±0.1Ba	2.6±0.1Ab

(4) 40°C recovery temperature (incubation temperature after HHP)

HHP	400 MPa for cells grown at temp. of				600 MPa for cells grown at temp. of			
Tr. Temp.	15°C	25°C	35°C	40°C	15°C	25°C	35°C	40°C
Recovery Cond.: aerobic. Stage: Late.								
4°C	3.5±0.1Aa	3.2±0.2Aab	2.9±0.2Ab	2.2±0.3Ac	5.4±0.1Aa	4.3±0.4Ab	4.2±0.4Ab	4.2±0.2Ab
20°C	3.5±0.1Aa	3.0±0.3ABb	2.7±0.2Abc	2.3±0.1Ac	5.1±0.2Ba	4.5±0.4Aab	4.4±0.2Ab	4.3±0.1Ab
40°C	4.0±0.2Ba	3.8±0.5Ba	3.7±0.3Ba	3.4±0.2Ba	5.8±0.1Ca	5.5±0.3Bab	5.3±0.2Bb	5.1±0.2Bb
Recovery Cond.: anaerobic. Stage: Late.								
4°C	3.4±0.2Aa	3.2±0.1Aa	2.9±0.3Aab	2.4±0.4Ab	5.4±0.2Aa	4.3±0.5Ab	4.3±0.4Ab	4.1±0.3Ab
20°C	3.4±0.1Aa	3.0±0.1ABab	2.8±0.4Ab	2.5±0.2Ab	5.1±0.1Aa	4.7±0.3ABab	4.4±0.3Ab	4.3±0.3Ab
40°C	4.0±0.2Ba	3.7±0.4Bab	3.8±0.2Bab	3.4±0.1Bb	5.8±0.2Ba	5.4±0.2Bab	5.5±0.2Bab	5.2±0.2Bb
Recovery Cond.: aerobic. Stage: Early.								
4°C	1.5±0.2Aa	1.2±0.1Aa	1.5±0.1Aab	0.9±0.2Ab	2.8±0.2Aa	2.6±0.3Aab	2.9±0.2Aab	2.3±0.2Ab
20°C	1.3±0.2Aa	1.0±0.3Aab	1.4±0.2Aab	0.8±0.2Ab	3.0±0.2ABa	2.7±0.2Aab	3.1±0.3ABab	2.6±0.1Ab
40°C	2.6±0.2Ba	2.3±0.2Ba	2.4±0.3Ba	1.7±0.1Bb	3.7±0.5Ba	3.0±0.3Aa	3.4±0.1Ba	3.0±0.0Ba
Recovery Cond.: anaerobic. Stage: Early.								
4°C	1.5±0.4Aa	1.1±0.2Aab	1.6±0.1Aab	1.0±0.2Ab	2.8±0.3Aa	2.6±0.2Aab	3.1±0.2Ab	2.5±0.1Ab
20°C	1.3±0.3Aa	1.0±0.1Aa	1.4±0.3Aa	1.1±0.2Aa	3.2±0.3Aa	2.7±0.1Aa	3.2±0.4Aa	2.8±0.1Ba
40°C	2.4±0.4Ba	2.3±0.3Ba	2.3±0.2Ba	1.8±0.1Ba	3.5±0.5Aa	3.0±0.2Aa	3.4±0.1Aa	3.2±0.1Ca

Chapter 5

FUTURE RESEARCH

In summary, the use of antimicrobial coatings and high pressure processing can be used in order to delay or reduce the populations of *L. monocytogenes*, *E. coli* O157:H7, and *Salmonella* spp. However, when using pressure for pathogen reduction in food, several factors must be carefully considered and controlled in regards to the storage conditions, treatments conditions, and temperature history of the product. One possible future area of research could be studying the effects of the bacteria being grown on food versus the bacteria being grown in a nutrient medium in regards to high pressure processing. The surprising results concerning the effect of growth phase of the bacteria on high pressure show that it could be possible there is some difference between the pressure resistance of L. monocytogenes, E. coli, and Salmonella when grown in a nutrient medium as opposed to as in a food matrix. Another possible area that could be explored with this research is to see if a larger log reduction could be attained through a "multiple hurdle" strategy by combination of antimicrobial coatings and high pressure processing. The use of antimicrobials is anticipated to further inhibit the outgrowth of pathogens during storage of refrigerated and processed foods of extended durability (REPFED) such as roasted turkey. In addition, the combined use of HHP and modified atmosphere packaging (MAP) could be another area of future research.