

EFFECT OF INTRINSIC FACTORS ON GROWTH OF *LISTERIA*  
*MONOCYTOGENES* IN SLICED DELI TURKEY

by

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## Abstract

Intrinsic factors impact *Listeria monocytogenes* growth in ready-to-eat poultry products. Sliced deli turkey was formulated with in-going concentrations of 1.5% NaCl or 0.75% NaCl/0.75% KCl, 0 ppm or 200 ppm NaNO<sub>2</sub>, and using 10% or 45% pump for a total of 8 treatments. Turkey roasts were sliced and inoculated with a 5-strain *L. monocytogenes* cocktail or peptone water (control), vacuum packaged, and stored at 4°C. Treatments were sampled on days 0, 7, 14, 21, 28, 42, 63, and 91 of storage to determine *L. monocytogenes* mean log growth and aerobic plate count (APC). The pH, water activity, residual nitrite concentration, and percent fat, moisture, protein, and sodium were measured using control treatments on each sampling day. There was a nitrite by day and a percent pump by day interaction (P<0.05) for *L. monocytogenes* and APC populations. *Listeria monocytogenes* populations in treatments containing 200 ppm NaNO<sub>2</sub> were 0.70 to 2.39 log CFU/cm<sup>2</sup> lower compared with products formulated with 0 ppm NaNO<sub>2</sub>. Using 10% pump reduced *L. monocytogenes* populations by 0.62 to 1.50 log CFU/cm<sup>2</sup> on days 7 to 28 and at day 63 compared with 45% pump treatments. Incorporating 1.5% NaCl or 0.75% NaCl/0.75% KCl into formulations did not affect (P>0.05) *L. monocytogenes* populations during storage. On days 7 through 91, APC populations were 0.76 to 2.96 log CFU/cm<sup>2</sup> lower with inclusion of 200 ppm NaNO<sub>2</sub> compared to 0 ppm NaNO<sub>2</sub>. There was a treatment by day interaction (P<0.05) for *L. monocytogenes* populations and APC. The initial inoculum level of *L. monocytogenes* averaged 2.21 log CFU/cm<sup>2</sup> and was similar (P>0.05) for all treatments on day 0. *Listeria monocytogenes* populations increased (P<0.05) from day 0 to 14 by 1.30 to 5.04 log

CFU/cm<sup>2</sup>. Overall, *L. monocytogenes* populations increased during storage and by day 91 *L. monocytogenes* populations were similar regardless of NaNO<sub>2</sub> level used except for treatments formulated with 0.75% NaCl/0.75% KCl and 10% pump. *Listeria monocytogenes* and APC populations were influenced by nitrite concentration and percent pump, while inclusion of NaCl or NaCl/KCl did not affect *L. monocytogenes* growth during refrigerated storage in vacuum packed sliced deli turkey.

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## Chapter 1 - INTRODUCTION

The presence of *Listeria monocytogenes* in ready-to-eat (RTE) meat and poultry products is a major safety concern for the food industry as it has the ability to grow at refrigeration temperatures. *L. monocytogenes* is an important foodborne pathogen, estimated to cause approximately 1,662 illnesses, 1,520 hospitalizations, and 266 deaths each year in the U.S. (Scallan and others 2011b). Due to the ubiquity of *L. monocytogenes* in the environment, it is challenging to control transmission of this organism from raw animal products in the meat-processing environment to RTE meat products (Tompkin and others 1999). While the lethality treatment received by RTE products kills *L. monocytogenes*, products may become re-contaminated post-thermal processing (USDA FSIS 2006). To help control *L. monocytogenes* contamination in RTE meat and poultry products, the United States Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) (2006) established three alternatives that processors use to minimize post-lethality exposure.

In 1999, a multi-state outbreak of listeriosis linked with consumption of precooked RTE turkey deli meat affected 54 persons, caused eight deaths, and three fetal deaths (CDC 1999). In addition, a multi-state outbreak of *L. monocytogenes* in 2002 was linked to eating sliceable turkey deli meat that resulted in 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states. In a report by Levine and others (2001), cooked poultry products had a *L. monocytogenes* prevalence of 2.12%. A risk assessment undertaken by the Food and Drug Administration (FDA), USDA FSIS, and the Centers for Disease Control and Prevention (CDC) categorized deli meats as

having the highest risk level for serious illness and death associated with consumption of RTE foods potentially contaminated with *L. monocytogenes* on a per serving and per year basis (U.S. Dept of Health 2003). Between 1990 and 1999, 4.2% to 8.0% of sliced luncheon meat samples were positive for *L. monocytogenes* out of approximately 2,300 samples collected by FSIS from U.S. federally inspected establishments (Levine and others 2001).

Intrinsic factors play a major role in the ability for *L. monocytogenes* to grow and survive in meat and poultry products. Factors such as sodium nitrite ( $\text{NaNO}_2$ ), percent pump, and sodium chloride ( $\text{NaCl}$ ) or the combination of  $\text{NaCl}$  and potassium chloride ( $\text{KCl}$ ) content have the potential to influence growth of *L. monocytogenes*. Sodium nitrite significantly reduces *L. monocytogenes* growth in vacuum-packed cooked meats stored at  $5^\circ\text{C}$ . In addition,  $\text{NaNO}_2$  increases the lag phase of *L. monocytogenes* (Duffy and others 1994). In a study by Seman and others (2002), increased finished-product moisture increased *L. monocytogenes* growth rates. *Listeria monocytogenes* is a salt-tolerant organism, and some strains can grow in 12.0%  $\text{NaCl}$  solutions (Galdiero and others 1997, and Gnanou and others 2000). Therefore, the presence of these intrinsic factors play a major role in the growth of *L. monocytogenes* in meat and poultry products.

The objective of this study was to evaluate how the combination of  $\text{NaNO}_2$  concentration, percent pump, and  $\text{NaCl}$  or a blend of  $\text{NaCl}/\text{KCl}$  affect the growth of *L. monocytogenes* in vacuum packaged sliced deli turkey stored at  $4^\circ\text{C}$  for up to 91 days.

## **Chapter 2 - REVIEW OF LITERATURE**

### **Turkey Industry**

During the last two decades, the turkey industry has evolved from a single-product, holiday-oriented business into a fully integrated industry with diversified product lines that compete year-round with other protein products. Due to the nutritional value and flavor of turkey, consumption has increased 109% since 1970. The per capita consumption of turkey has increased from 3.7 kg per person in 1970 Ward (2004) to 7.7 kg per person in 2009 (National Turkey Federation 2010). By processing turkey into a wide range of products, the total value of further processed turkey products was more than \$13.9 billion in 2007 (National Turkey Federation 2010). In 2009, turkey was the fourth ranked protein of choice for United States (U.S.) consumers (National Turkey Federation 2010).

According to the National Turkey Federation's 2009 Marketplace Survey, 41.1% of turkey products were distributed to grocery stores and other retail outlets; 27.9% were distributed as a commodity; 17.7% went to foodservice outlets, and 6.4% were exported. The top three turkey products sold in 2009 were whole birds, deli meat, and ground turkey. In a 2009 report by the American Meat Institute (AMI), expenditures on turkey products increased from \$18.52 in 1997 to \$19.92 in 2007. Meat and poultry expenditures accounted for 1.7% of disposable income per person, and 29.7% of total food expenditures (AMI 2009). According to Kreuser (2008), turkey and ham were the top deli meat flavors and each accounted for over 30% of all deli meat dollar sales during a 52-

week period. The trend for increased turkey consumption is expected to continue in the future as the deli-meat industry continues to thrive.

### ***Listeria monocytogenes***

*Listeria monocytogenes* was discovered over 80 years ago by E.G.D. Murray and James Pirie (Murray and others 1926). Murray, from Johannesburg, South Africa originally named it *Bacterium monocytogenes* because of a characteristic monocytosis found in infected laboratory rabbits and guinea pigs. *Listeria monocytogenes* is a member of the family Corynebacteriaceae, order Eubacteriales. It is an ubiquitous gram-positive, non-spore forming, short rod (0.4-0.5 x 0.5-2.0  $\mu\text{m}$ ), and motile by flagella (Bell and Kyriakides 2005). It is able to survive and multiply at temperatures around 0°C in the presence or absence of oxygen. *Listeria monocytogenes* tolerates up to 12-13% NaCl, grows at a water activity ( $a_w$ ) down to 0.92, and at a pH of 4.4 to 9.4. The optimum temperature for *L. monocytogenes* growth is approximately 37°C and the optimum pH for growth is approximately 7.0 (Bell and Kyriakides 2005). The optimum  $a_w$  for the growth of *L. monocytogenes* is approximately 0.97. It can survive for extended periods under harsh environmental conditions and then recover when introduced into a food as a cross-contaminant (Bell and Kyriakides 2005).

*Listeria monocytogenes* causes listeriosis which affects primarily pregnant women, the elderly, and persons with weakened immune systems. As a result of foodborne outbreaks, interest in *L. monocytogenes* grew in the 1980's among food manufacturers and government agencies (Cossart and Mengaud 1989; Lamont and others 1988). *Listeria monocytogenes* is a widespread problem in public health and for food industries. The infectious dose of *L. monocytogenes* is unknown but is believed to vary

with the strain and susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, it is safe to assume that in susceptible persons, fewer than 1,000 total organisms may cause disease (USDA FSIS 2009b). Since 1987, the FSIS initiated regulatory microbiology testing for *L. monocytogenes* in RTE meat and poultry products mandating a “zero tolerance” (no detectable level permitted) (USDA FSIS 2000). The first reported foodborne outbreak of listeriosis in North America was linked to coleslaw in Nova Scotia in 1980-1981. There were 41 cases of listeriosis recorded from that outbreak that resulted in 18 deaths: two adults and 16 fetal or newborn infants (Bell and Kyriakides 2005). Ready-to-eat foods appear to represent the main source of sporadic foodborne listeriosis infections and have been implicated as the source in a number of listeriosis outbreaks (Amezquita and Brashears 2002; Lianou and others 2007).

### **Turkey Microbiology**

Due to the ubiquity of *L. monocytogenes* in the environment, it is challenging to control transmission of this organism from raw animal products in the meat-processing environment to RTE meat products (Tompkin and others 1999). Poultry harbors a very complex microflora which is partly of intestinal origin due to the production system, flocks of large numbers of fast growing animals, and being reared in climatized houses on litter floors (Bolder 1998). In a report by Levine and others (2001), cooked poultry products had a *Salmonella* prevalence of 0.10%, and 2.12% prevalence of *L. monocytogenes*. The nationwide young turkey microbiological baseline data collection program (USDA FSIS 1998) evaluated 1,221 turkey carcasses and recovered 90.3% *Campylobacter jejuni/coli*, 66.7% *Staphylococcus aureus*, 29.2% *Clostridium*

*perfringens*, 18.6% *Salmonella*, and 5.9% *L. monocytogenes*. In 2009, USDA compared changes in percent positive rates of *Salmonella* and *Campylobacter* between re-hang and post-chill on turkey carcasses. *Salmonella* populations were reduced from 4.99% to 0.35% due to processing steps between re-hanging and post-chilling while *Campylobacter* was reduced from 22.68% to 1.11%, suggesting that anti-microbial interventions were effective (USDA FSIS 2009a).

*Listeria monocytogenes* can increase to high numbers in cured or noncured RTE meats during refrigerated storage due to its ability to survive and grow under low temperatures (Lou and Yousef 1999). Prior to the USDA FSIS (2003) Interim Final Rule entitled “Control of *Listeria monocytogenes* in RTE Meat and Poultry Products,” surveillance and monitoring activities by FDA and USDA indicated that as much as 5% of certain RTE foods such as prepared deli-style salads and sliced luncheon meats contained *L. monocytogenes* (Hitchins 1996; Levine and others 2001). In 2005, the Foodborne Illness Active Surveillance Network (FoodNet) recorded a total of 16,614 laboratory-confirmed cases for pathogens, and of these, *Listeria* was responsible for 135 cases with an overall incidence level of 0.30 per 100,000 population (CDC 2006). Many RTE products serve as a good host for the growth of *L. monocytogenes* because they are stored under refrigeration temperatures in anaerobic environments, could potentially be contaminated through post-processing handling, and are often a high moisture product resulting in parameters that are favorable for growth.

Naturally occurring bacterial counts in oven-roasted sliced turkey hams and breast rolls have been found to be 2.72 and 6.22 log CFU/cm<sup>2</sup>, respectively (Zhu and others 2008). In a study by Samelis and Metaxopoulos (1999) evaluating the presence of

*Listeria*, sliced vacuum packed cooked meats were contaminated with 23.3% *Listeria* spp. and 6.7% *L. monocytogenes* while country-style sausage contained 40% and 10%, respectively.

In general, populations of *L. monocytogenes* in processed meat products are low, with 80-90% of samples containing fewer than 10 to 100 CFU/g (Marsden and others 2001). Higher populations have been documented in RTE products, particularly deli products, hotdogs, sausages, and luncheon meats. These products have been associated with *L. monocytogenes* outbreaks (Rocourt and Cossart 1997). Post-processing contamination continues to be a concern for RTE meats since *L. monocytogenes* is a ubiquitous organism that can be an environmental contaminant.

### **Boneless Breast Trimmings**

Products such as boneless breast trimmings and ground turkey meat are used in the production of deli turkey roasts. “Boneless poultry breast trimmings are defined as trimmings that are removed from the breast portion only. When a product is formulated with boneless breast trimmings, the amount of skin should be indicated in order to determine that the meat requirement is met for a standardized product and that the product is properly labeled. Trimmings from the ribs may be identified as white turkey or white chicken trimmings, or white turkey or white chicken rib meat (excluding skin)” (USDA FSIS 2005). Boneless breast trimmings are widely used in the production of deli turkey roasts due to their ability to bind well and provide adequate flavor and nutritional value.

## **Public Health Impact of *Listeria monocytogenes***

Foodborne agents cause an estimated 9.4 million illnesses annually in the U.S. (Scallan and others 2011a). *Listeria monocytogenes* is an important foodborne pathogen, estimated to cause approximately 1,662 illnesses, 1,520 hospitalizations, and 266 deaths in the U.S. each year (Scallan and others 2011b). According to preliminary surveillance data available from the CDC and FoodNet, the overall incidence of listeriosis in the U.S. in 2005 was 3.0 cases per million people (CDC 2006), while the corresponding incidence in 2004 was 2.7 cases (CDC 2005). Listeriosis is a serious infection caused by eating food contaminated with the bacterium *L. monocytogenes* which has been recognized as an important public health problem in the U.S. (CDC 2002).

A 2003 risk assessment conducted on 23 categories of RTE foods indicated that deli meats posed the greatest risk for listeriosis, accounting for approximately 1,600 illnesses per year (USDA FSIS 2010). In 1999, a multi-state outbreak of listeriosis linked with consumption of precooked, RTE turkey deli meat affected 54 persons, causing eight deaths, and three fetal deaths (CDC 1999). In 2002, another multi-state outbreak of *L. monocytogenes* linked to eating sliceable turkey deli meat resulted in 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states. Listeriosis is and has been of great concern to public health (CDC 2002).

## **Post-Processing Contamination**

*Listeria monocytogenes* contamination is a concern in RTE meat and poultry products. While the lethality treatment received by RTE products kills *L. monocytogenes*, products may become re-contaminated post-thermal processing (USDA FSIS 2006). Post-processing contamination is of concern to processors as it can cause human illness

or death, loss of product, and be an economical burden. Based on a report by Lin and others (2006), the processing plant environment appears to be a greater source of *L. monocytogenes* contamination for finished product than raw materials. In addition, *L. monocytogenes* can adhere to most materials that make up food processing equipment, including equipment used in deli meat plants (Beresford and others 2001, and Lunden and others 2003). Therefore, the presence of *L. monocytogenes* in cooked meat products is most likely the result of recontamination following thermal processing which may occur during post-thermal processing handling procedures such as peeling, slicing, and repackaging (Johnson and others 1990, Tompkin 2002, and U.S. Dept of Health 2003). In a study by Lin and others (2006) on the detection of *L. monocytogenes* on oven-roasted turkey, salami, and beef bologna, the greatest number of positive samples were obtained from oven-roasted turkey following storage at 4°C. In a risk assessment undertaken by FDA, USDA FSIS, and the CDC, deli meats were categorized as having the highest level for risk of serious illness and death associated with consumption of RTE foods potentially contaminated with *L. monocytogenes* on both a per serving and per year basis (U.S. Dept of Health 2003).

### **Deli Turkey Product and *L. monocytogenes***

Foodborne illnesses are a substantial health burden to the U.S. economy and a financial burden to the food industry. Precooked RTE products such as deli turkey provide a favorable host for *L. monocytogenes* which is a psychrotroph and can multiply and grow during refrigerated storage. Deli turkey has a pH around 6.3 (Glass and Doyle 1989) and an  $a_w$  around 0.96 - 0.993 (Duffy and other 1994) that falls within the range for *L. monocytogenes* growth and survival. A major multistate outbreak linked to hot dogs

and deli meats occurred in 1998-1999. This resulted in at least 50 illnesses reported in 11 states caused by a strain of *L. monocytogenes*. Six adults died and two pregnant women had spontaneous abortions (CDC 1999). In a study by Gombas (2003), 9,199 samples of luncheon meat tested for *L. monocytogenes* contamination were found to have 0.89% positive samples. This was lower than reported rates of 4.2% to 8.0% for sliced luncheon meat out of approximately 2,300 samples collected by USDA FSIS from U.S. federally inspected establishments between 1990 and 1999 (Levine and others 2001) and about 6% for sliced cooked ham and poultry products out of approximately 900 samples collected from Belgium supermarkets in 1996 and 1998 (Uyttendaele and others 1999).

Studies have demonstrated positive *L. monocytogenes* contamination on retail frankfurters and luncheon meats in the U.S. Marsden (1994) indicated that 1.4%, or 294 out of 20,296 samples of processed meats harbored *L. monocytogenes*. Twenty retail frankfurter brands evaluated by Wang and Muriana (1994) were found to be contaminated at a rate of 7.5% with six out of the 20 sampled brands positive for *L. monocytogenes*. Cooked RTE poultry products may demonstrate even higher frequencies and levels of contamination (Farber and Peterkin 2000). Equipment for further processing handles a high volume of products and can contribute to the cross contamination of products (Bolder 1989). Turkey products contaminated with *L. monocytogenes* is a huge concern for the public and the food industry as it corresponds to human illness, tremendous costs, and billions of kilograms of recalled product.

### **FSIS *Listeria* Compliance Guidelines**

*Listeria monocytogenes* is a ubiquitous pathogen that survives in cold damp environments. Because *L. monocytogenes* may be present in slaughter facilities, in raw

meat and poultry, as well as in other ingredients used in processed meat products, it can be continuously introduced into the processing environment. Thermal processing of RTE meat and poultry products generally eliminates *L. monocytogenes*, however products can be recontaminated after the lethality treatment by exposure during slicing, repackaging, cutting, peeling and other procedures. To control *L. monocytogenes* contamination in RTE meat and poultry products, USDA FSIS (2006) established three alternatives processors can use to minimize post-lethality exposure.

Under Alternative 1, the establishment must use a post-lethality treatment to reduce or eliminate *L. monocytogenes*, and an antimicrobial agent or process to suppress or limit the growth of the pathogen. In addition to Alternative 1, the establishment must provide validation of the post-lethality treatment as being effective in eliminating or reducing *L. monocytogenes* to an undetectable level, and the validation should specify the log reduction or suppression achieved by the post-lethality treatment and antimicrobial agents. Establishments under Alternative 1 are subject to the lowest frequency of testing by USDA FSIS.

An establishment producing products under Alternative 2 must use either a post-lethality treatment or an antimicrobial agent or process. If a processor uses the post-lethality treatment, they must also provide validation as in Alternative 1. If the establishment chooses to use an antimicrobial agent or process, it must validate its effectiveness and include that information in their HACCP plan. Establishments falling under Alternative 2 are subject to more frequent USDA FSIS verification than Alternative 1.

Under Alternative 3, an establishment relies on its sanitation program to control *L. monocytogenes* in RTE meat and poultry products. An establishment operating under Alternative 3 is subject to the most frequent USDA FSIS verification testing compared to Alternatives 1 and 2. Like all RTE products exposed to the processing environment, deli and hotdog products that are exposed to the post-processing environment are subject to this rule. Depending on the method that an establishment chooses to control *L. monocytogenes* in the post-lethality environment products are regulated under Alternative 1, 2, or 3.

### **USDA FSIS Lethality and Stabilization Guidelines**

A 1999 final rule published in 9 CFR 381.150 USDA FSIS (1999c) established a performance standard for poultry that requires a 7.0 log lethality reduction of *Salmonella* in RTE poultry. Appendix A (USDA FSIS 1999b) is the “Compliance Guidelines for Meeting Lethality Performance Standards for Certain Meat and Poultry Products” for the reduction of *Salmonella*. In an effort to assist establishments to meet these lethality standards, USDA FSIS issued this compliance guideline that is based upon time and temperature requirements. According to Appendix A, cooked poultry rolls and other cooked poultry products should reach an internal temperature of at least 71.1°C instantaneously prior to being removed from the cooking medium. Cured and smoked poultry rolls and other cured and smoked poultry should reach an internal temperature of at least 68.3°C instantaneously prior to being removed from the cooking medium. Cooked RTE product to which heat will be applied incidental to a subsequent processing procedure may be removed from cooking for such processing provided that it is immediately fully cooked to the 71.1°C internal temperature. Establishments producing cooked poultry rolls and other cooked poultry products should have sufficient monitoring

equipment, including recording devices, to assure that the temperature limits of these processes are being met. USDA FSIS requires that accuracy of the temperature recording device to be within  $\pm 1.8^{\circ}\text{C}$ .

Appendix B (USDA FSIS 1999a) describes the “Compliance Guidelines for Cooling Heat-Treated Meat and Poultry Products (Stabilization).” Products are required to meet stabilization performance standards under Appendix B for preventing the growth of spore-forming bacteria, particularly *Clostridium perfringens*. It is very important to cool the product in a timely manner and to avoid excessive time in the range of  $54.5^{\circ}\text{C}$  to  $26.7^{\circ}\text{C}$ , since this is the range for rapid growth of *C. perfringens*.

## **Intrinsic Factors Affecting *Listeria monocytogenes* Growth**

### **pH**

According to Bell and Kyriakides (2005), Gray and Killinger (1996), Abee and Wouters (1999), and Petran and Zottola (1989), optimum growth for *L. monocytogenes* is at a pH of approximately 7.0. Wehr (1987) stated that the pH growth range of *L. monocytogenes* is 5.0 – 9.6, but the organism can survive at lower pH values. However, Farber and Peterkin (1991) stated that *L. monocytogenes* can grow at pH values as low as 4.5. Growth of *L. monocytogenes* on processed meats is closely related to the pH of the product (Glass and Doyle 1989). In general, *L. monocytogenes* generally grows well on ham, bologna, wieners, sliced chicken, sliced turkey that have a pH near or above 6.0 and grows poorly or not at all on products with a pH near or below 5.0 (Glass and Doyle 1989). In a report by Duffy and others (1994), incorporation of sodium tripolyphosphate raised the pH of cooked beef, pork, chicken, and turkey, leading to an increased growth

rate of *L. monocytogenes*. Product pH is a factor that influences overall growth of *L. monocytogenes* in meat products.

### **Water Activity**

Water activity is the ratio of the vapor pressure of water in equilibrium with a food to the saturation vapor pressure of water at the same temperature. The  $a_w$  of a food describes the degree to which the water is “bound” in the food and influences its availability to act as a solvent, participate in chemical/biochemical reactions, and growth of microorganisms (Fontana 1998). The  $a_w$  of a food product is lowered by the addition of sodium chloride, sugars, and other solutes. The higher the concentration of the solute, the lower the  $a_w$  of the product. The optimum  $a_w$  for the growth of *L. monocytogenes* is approximately 0.97 (Bell and Kyriakides 2005). If the  $a_w$  in a product is lower than the minimum necessary for growth of *L. monocytogenes*, growth of the organism will be prevented or minimized provided the  $a_w$  of the product does not increase during the shelf life or use of the product.

Lou and Yousef (1999) studied the effect of different  $a_w$  levels on the survival and/or growth of *L. monocytogenes*. They found the minimum  $a_w$  was approximately 0.92 and 0.94, respectively, for growth of a variety of *L. monocytogenes* strains at 10 and 4°C when NaNO<sub>2</sub> was used. Food products with an  $a_w$  of <0.90 may confer a greater heat resistance on the organism than those at higher  $a_w$  levels around 0.98. Although *L. monocytogenes* does not grow at low  $a_w$  levels, it can survive for significant periods of time in foods with an  $a_w$  <0.90 that are stored at low temperatures (Bell and Kyriakides 2005). The effect of decreased  $a_w$  levels on microbial growth of microorganisms is an

extension of the lag phase, a suppression of the log phase, or a reduction in the total number of viable microorganisms (Troller1989).

In a study by Glass and Doyle (1989) and Buchanan and others (1989),  $a_w$  plays a role in limiting the growth of *L. monocytogenes*. Controlling the  $a_w$  of products is an important factor from a microbiological standpoint and for the stability and safety of food products. If  $a_w$  is less than the optimum 0.97 for growth of *L. monocytogenes*, growth will be inhibited due to less water in the product. In a report by Shahamet and others (1980), *L. monocytogenes* was able to survive more than 18 weeks in 25.5% NaNO<sub>2</sub> solution at 4°C which had an  $a_w$  lower than 0.90. They suggested that *L. monocytogenes* is very resistant to a lower  $a_w$ . Petran and Zottola (1989) reported that *L. monocytogenes* was unable to grow at an  $a_w$  less than 0.92 in varying levels of carbohydrates in tryptic soy broth (TSB). Water activity is an important factor that influences overall growth of *L. monocytogenes* and the stability and safety of meat products.

### **Sodium Nitrite**

When NaNO<sub>2</sub> is added to meat, nitric oxide metmyoglobin is formed resulting in a brown color. Heating causes the reaction to form a stable pink color known as nitrosylhemochromogen (Killday and others 1988) that contributes to flavor and prevents outgrowth of *Clostridium botulinum* in vacuum packaged meat products (MacDougall and others 1975). Duffy and others (1994) found that the incorporation of NaNO<sub>2</sub> significantly reduced the growth of *L. monocytogenes* in vacuum-packed cooked meats stored at 5°C. In addition, NaNO<sub>2</sub> increased the lag time, plus the lag time was further increased by the presence of sodium ascorbate. The addition of 0.042% sodium ascorbate to cured meat significantly increased the effectiveness of residual nitrite in inhibiting

growth of *L. monocytogenes* (Duffy and others 1994). Sodium nitrite exerts some inhibitory effects against *L. monocytogenes*, although it does not consistently prevent growth at the concentrations remaining in many cooked meat products. This anti-listerial effect is enhanced at pH levels as <6.0 (Lou and Yousef 1999). In a study conducted by Shahamat and others (1980) on the efficacy of NaNO<sub>2</sub> for the control of *L. monocytogenes*, they determined that its activity was dependent on interactions with temperature, pH, and NaCl content. They concluded that at levels permitted in meat products, NaNO<sub>2</sub> would have significant inhibitory activity only in refrigerated products containing at least 3% NaCl and having a pH of 5.5 or less. In support of NaNO<sub>2</sub> being dependent upon other factors, Buchanan and others (1989) stated that NaNO<sub>2</sub> was more effective against *L. monocytogenes* when the incubation temperature of the media was lowered to 5°C. Raising the level of NaCl from 0.5% to 4.5% also increased the inhibitory effect of NaNO<sub>2</sub> upon *L. monocytogenes*. They also stated that NaNO<sub>2</sub> displayed both bacteriostatic and bactericidal activity against *L. monocytogenes*. A study by Junttila and others (1989) indicated that NaNO<sub>2</sub> accelerated the inactivation of *L. monocytogenes* in fermented meats. Sodium nitrite has the ability to reduce the growth of *L. monocytogenes* in many meat and poultry RTE products depending on the use and its interactions with pH, temperature, NaCl, and other contributing factors.

## **Fat**

The growth of *L. monocytogenes* is dependent on intrinsic factors, including the fat content of the product. Studies reported by Jung and others (1992) and Mehta and Tatini (1994) stated that fat content may affect growth of *L. monocytogenes* in dairy products. Hu and Shelef (1996) evaluated the effect of fat content on the behavior of *L.*

*monocytogenes* in pork liver sausage batter at 4°C and 10°C with fat levels of 22%, 37%, 52%, and 67% by weight. *Listeria monocytogenes* populations in control samples with 22% fat were higher ( $P<0.05$ ) than samples with added lard; however, increasing the fat content in the sausage batter from 22% to 67% produced only small changes in increasing growth of *L. monocytogenes*. The differences between *L. monocytogenes* growth within sausage batters having a fat content of 22% to 67% was less than 1.5 CFU/g at each storage time.

Mehta and Tatini (1994) reported that fat reduction in cheddar cheese had no effect on the growth of *L. monocytogenes*. In a study by Barmpalia-Davis and others (2009), *L. monocytogenes* populations on 4.5% and 32.5% fat frankfurters exceeded 8.0 log CFU/g at 39 and 55 days of storage, respectively. Epidemiological investigation and studies with animal models have suggested an association between the fat content of foods and increased likelihood of foodborne listeriosis (Barmpalia-Davis and others 2009). However, the authors noted that information regarding the contribution of fat directly on the gastrointestinal survival of *L. monocytogenes* is limited. In this study, the findings indicated that the fat content of frankfurters appeared to protect *L. monocytogenes* against gastric acidity (Barmpalia-Davis and others 2009). Based on the studies reviewed, increased fat content decreased the growth of *L. monocytogenes* and extended the time of *L. monocytogenes* growth. Fat content is an important factor that has the ability to influence overall growth of *L. monocytogenes* in some meat products.

## **Moisture**

Moisture content has the ability to play an important role in the growth of *L. monocytogenes* in RTE meat and poultry products. Adding water to a product can

increase the  $a_w$  if solutes are not available to chemically bind water, leading to increased growth of *L. monocytogenes*. In a study by Seman and others (2002), increasing finished-product moisture from 45.5% to 83.5% had a trend to increase growth rates of *L. monocytogenes*. Conversely, reducing the moisture content in the product may lead to a reduction in  $a_w$  and affecting microbial growth by increasing the lag phase and decreasing the growth rate. Wijtzes and others (1993) stated that increasing the NaCl content and decreasing the moisture content will reduce product  $a_w$  which would reduce the growth rate and ultimately prevent growth of *L. monocytogenes*.

## **Protein**

Protein effects *L. monocytogenes* growth by the interaction with pH, NaCl content, moisture content, fat content,  $\text{NaNO}_2$ , and other intrinsic factors. The fate of *L. monocytogenes* during refrigerated storage on several processed meat products was evaluated by Glass and Doyle (1989). These included ham, bologna, wieners, sliced chicken, sliced turkey, fermented semidried sausage, bratwurst, and cooked roast beef. The products were surfaced inoculated and stored for up to 12 weeks at 4.4°C or until the product was spoiled (determined visually, gas formation in pouch and/or turbidity in exudate). *Listeria monocytogenes* survived, but did not grow, on fermented semidried sausage, grew only slightly on cooked roast beef, grew well on some wiener products with a pH of 6.2, but not on others that had a pH of 5.9, and had a  $10^3$  to  $10^4$  log CFU/g increase within 4 weeks on sliced chicken and turkey. It appears that the rate of growth depended largely upon the type of product and the pH which ranged from 4.8 to 6.5. The overall protein source contributed to the ability of *L. monocytogenes* to survive and grow on different products.

## Salt

Traditionally, NaCl has been used to preserve food products by inhibiting the growth of spoilage and pathogenic bacteria. *Listeria monocytogenes* is a salt-tolerant organism and some strains can grow in 12.0% NaCl or survive 150 days in pure NaCl at ambient temperatures (Galdiero and others 1997; Gnanou and others 2000). *Listeria monocytogenes* has been reported to grow in a 6.0% NaCl brine solution (Hudson 1992) and in meat bacto tryptone (MBT) peptone media containing 8.0% NaCl (Vasseur and others 1999). Wilson and Miles (1974) stated that *L. monocytogenes* can remain viable for a year in 16.0% NaCl, while Wramby (1944) found that *L. monocytogenes* was resistant to 20.0% NaCl for a period of eight weeks when incubated at 4°C. Shahamat and others (1980) found that *L. monocytogenes* could survive for more than 18 weeks in 25.5% NaCl. In a study by Duche and Labadie (2003), the growth of *L. monocytogenes* was not influenced when grown in media containing up to 3.0% NaCl. Slow growth was observed in concentrations ranging from 4.0% to 6.0% NaCl, which disagrees with previous studies by Galdiero and others (1997) and Vasseur and others (1999). Duche and Labadie (2003) found that when concentrations of NaCl were above 8.0%, growth of *L. monocytogenes* ceased after 10 h in a lyophilized methionine-poor medium.

In recent years, there has been a tendency to reduce sodium in food because of its relationship with hypertension (Boziaris 2006). Processors have sought to address this and one strategy has been to partially replace NaCl with KCl or other salts. According to Boziaris and others (2006), the growth of *L. monocytogenes* in both NaCl and KCl were almost identical in terms of growth rate, death rate, lag phase duration, and maximum population. Sodium chloride can be replaced by KCl without risking the microbiological

safety of products up to a point. Conversely, the safety of the product is not enhanced by using KCl in place of NaCl. The ability of *L. monocytogenes* to survive under harsh NaCl conditions is one of the main reasons *L. monocytogenes* is difficult to control in many food products. According to Taormina (2010), NaCl is among the most efficacious ingredient in regard to its preservation properties against foodborne pathogens and spoilage organisms, and has the greatest impact on the microbiological safety and quality of foods.

### **Turkey Shelf Life Studies**

Shelf life of products plays an important role in the products ability to be suitable for food consumption and safe for the consumer. There is also high importance on preventing post-processing contamination of RTE meat products with *L. monocytogenes* (Glass and Doyle 1989). In a study by Zhu and others (2008), the growth of *L. monocytogenes* at 4°C over 28 days on vacuum packaged turkey hams inoculated at  $10^6$  to  $10^7$  log CFU/cm<sup>2</sup> and stored at 4°C was evaluated over 28 days. Populations increased about one log during the first seven days of storage at 4°C, then the organisms remained at  $10^7$  to  $10^8$  log CFU/cm<sup>2</sup> during the remainder of the storage period. During 28 days of refrigerated storage, aerobic plate counts (APC) on vacuum packaged turkey hams increased to 7.34 log CFU/cm<sup>2</sup>.

According to Pal and others (2008), *L. monocytogenes* took 16.9, 2.2, or 1.7 days to increase by 2 logs in sliced turkey breast at 4°C, 8°C, and 12°C, respectively. In addition, Pal and others (2008) showed that it would take less than 60 days to reach a 100-fold increase in *Listeria* in sliced turkey breast at 8°C and 12°C even with an initial load of 0.04 CFU/g. Lianou and others (2007) investigated the growth of *Listeria* in

vacuum packaged turkey breast at several time periods when stored at 4°C followed by aerobic storage at 7°C for 12 days to simulate consumer handling and poor refrigeration conditions. Their results showed that *L. monocytogenes* reached more than 10<sup>7</sup> log CFU/cm<sup>2</sup> from initial levels of 40 CFU/cm<sup>2</sup> within 12 days when sliced products were aerobically stored at 7°C. Glass and Doyle (1989) evaluated the fate of *L. monocytogenes* in vacuum packaged sliced turkey during refrigerated storage at 4.4°C for up to 12 weeks of storage or until the product was spoiled. *Listeria monocytogenes* grew on the sliced turkey with an increase of 2 logs CFU/g within 4 weeks (Glass and Doyle 1989). Growth was most prolific on processed poultry products compared to sliced chicken, roast beef, bratwurst, semidried summer sausage, and wieners. A study by Farber and Daley (1994) found that during four weeks of storage at 4°C, *L. monocytogenes* populations increased an average of 2.32, 2.12, 1.58, or decreased -0.58 logs CFU/g on turkey roll slices packaged in air, 30% CO<sub>2</sub> and 70% N<sub>2</sub>, 50% CO<sub>2</sub> and 50% N<sub>2</sub>, and 70% CO<sub>2</sub> and 30%N<sub>2</sub>, respectively. Mataragas and Drosino (2007) suggested that the end of quality based on shelf life can precede the time for *L. monocytogenes* to grow to the microbiological criterion for RTE meat products and that consumers would likely discard a product before *L. monocytogenes* can reach a risk level. Shelf life of meat products is an important factor, as it is important to provide the consumer with a safe and wholesome product.

### **Enumeration Media and Recovery of Injured Cells**

Modified oxford medium (MOX) base is a selective medium used for isolation of *Listeria* spp. Modified oxford medium base is prepared according to a formulation by Curtis and others (1989) who originally described the medium and its use for the

selective isolation of *Listeria* from mixed cultures. Peptone and beef heart digest provide nitrogen, carbon, amino acids, and vitamins (Difco 2009). Ferric ammonium citrate aids in the differentiation of *Listeria* spp. and hydrolyzes esculin so the addition of ferric ions to the medium detects this reaction. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6, 7-dihydroxycoumarin which reacts with ferric ions (Fraser and Sperber 1988).

Selectivity is provided by the presence of lithium chloride in the formula and is increased by adding various antimicrobial agents to the base. Incorporating these agents into MOX will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation. The most widely recognized antimicrobial agent combination is the Oxford medium formulation (Lee and McClain 1989) that contains cycloheximide, colistin sulfate, acriflavine, cefotetan, and fosfomycin (available as Oxford Antimicrobial Supplement). The modified Oxford medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobial Supplement) (Difco 2009).

Modified Oxford medium is recommended for isolating and identifying *L. monocytogenes* from processed meat and poultry products (Lee and McClain 1989). Oxford medium is recommended for isolating *Listeria* from enrichment broth cultures (Chesemore 1990). Kang and Fung (2000) developed the Thin Agar Layer (TAL) method to recover-heat injured *L. monocytogenes*, *Salmonella Typhimurium*, and *Escherichia coli* O157:H7 and to obtain good selectivity and recovery. After solidification of the sterilized selective agar in a petri dish, melted tryptic soy agar (TSA) is overlaid and allowed to solidify. This is followed by a second overlay of TSA. Preliminary studies by Wu and

Fung (2001) indicated that two thin layers of TSA agar worked better than one thicker layer of TSA agar for slowing down the selective agents in the selective medium that migrate through the TSA during the resuscitation period. Wu and Fung (2001) found no difference in *L. monocytogenes* growth between TSA and TAL, and they recovered higher numbers of cells using TSA or TAL compared to MOX. When evaluating heat-injured *L. monocytogenes* on TAL/MOX and MOX agar, the TAL/MOX system recovered about 1 log higher of heat-injured cells than MOX (Wu and Fung 2003). The basic principle of the TAL methodology is to have the injured cells resuscitate to a healthy cell during the migration of the selective agents to the top of the thin agar level.

### **Mechanism of Sodium Chloride**

Sodium chloride is perhaps the most effective and versatile antimicrobial ingredient used in foods and remains one of the most effective tools for the development of safe and wholesome food products (Taormina 2010). Functions of sodium in foods and beverages are as an essential nutrient, flavor modifier, preservative, and leavening agent (Miller 1996). Sodium in the form of NaCl has many important technological and processing contributions, such as altering meat and moisture binding in processed meat products (Desmond 2006). It is clear that NaCl plays a critical role controlling microbial growth, particularly in RTE foods. Sodium chloride has both a bacteriostatic effect and dehydrating effect on protein. Factors involved in preservation properties of NaCl include the direct toxicity of  $\text{Cl}^-$ , removal of oxygen from the medium, sensitization of the organisms to  $\text{CO}_2$ , and interference with the rapid action of proteolytic enzymes. Csonka (1989) reviewed osmotic regulation in bacteria and described the hyperosmotic shock imposed on cells by NaCl. Hyperosmotic shock causes shrinkage of the

cytoplasmic volume, a process known as plasmolysis. According to Shelef and Seiter (1993) the lowering of  $a_w$  is most likely the primary cause for microbial growth inhibition of NaCl. The efficacy of NaCl against growth or survival of microorganism in food systems is subject to many interactions with intrinsic and extrinsic factors. Sodium chloride is among the most efficacious in regard to the preservation properties against foodborne pathogens and spoilage organisms, and therefore has the greatest impact on the microbiological safety and quality of foods.

### **Mechanism of Sodium Nitrite**

Sodium nitrite acts primarily as an inhibitor for some microorganisms. Sodium nitrite exerts some inhibitory effects against *L. monocytogenes*, although it does not prevent growth at concentrations remaining in many cooked meat products (Lou and Yousef 1999). The search for an effective nitrite replacement has been complicated by the fact that the antibotulinal mechanism of nitrite remains unknown, although several mechanisms have been proposed (Yarbrough and others 1980; Tompkin and other 1978; Woods and others 1981).

Yarbrough and others (1980) concluded that nitrite inhibits bacteria by several mechanisms. First, nitrite interferes with energy conservation by inhibiting oxygen uptake, oxidative phosphorylation, and proton-dependent active transport. Second, nitrite acts as an uncoupler, causing a collapse of the proton gradient. Third, certain metabolic enzymes (aldolase) are inhibited. In addition Tompkin and others (1978) suggested that nitrite may react with ferredoxin, an iron-containing enzyme necessary for energy production in some clostridial vegetative cells, thus inhibiting growth.

Woods and others (1981) found that the addition of nitrite to a suspension of *C. sporogenes* in a glucose medium caused a rapid decrease in intracellular adenosine triphosphate (ATP) concentration and an accumulation of pyruvate in the medium. The accumulation of pyruvate was the result of inhibition of the phosphoroclastic oxidoreductase. They also concluded that, although nitric oxide can react with ferredoxin *in vitro*, the main site of inhibition within intact cells is the reaction of nitric oxide with the enzyme pyruvate-ferredoxin oxidoreductase. Sodium nitrite is widely used as an additive in cured meats where it has important antimicrobial and antitoxigenic properties.

## Chapter 3 - Sliced Deli Turkey

### Introduction and Objectives

The presence of *L. monocytogenes* in RTE meat and poultry products provides a major safety concern in the food industry as it has the ability to grow at refrigerated temperatures. *Listeria monocytogenes* is an important foodborne pathogen estimated to cause approximately 1,662 illnesses, 1,520 hospitalizations, and 266 deaths each year in the U.S. (Scallan and others 2011b). In 2002, a multi-state outbreak of *L. monocytogenes* linked to eating sliceable turkey deli meat resulted in 46 culture-confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states (CDC 2002). Since 1987, the FSIS initiated regulatory microbiology testing for *L. monocytogenes* in RTE meat and poultry products mandating a “zero tolerance” (no detectable level permitted) (USDA FSIS 2000). To help control *L. monocytogenes* contamination in RTE meat and poultry products, USDA FSIS (2006) established three alternatives processors can use to minimize post-lethality exposure.

Intrinsic factors play an important role on a product’s ability to support growth of *L. monocytogenes*. Sodium nitrite is one intrinsic factor that has been shown to reduce the growth of *L. monocytogenes* in RTE meat products. Duffy and others (1994) found that NaNO<sub>2</sub> increased the lag time of *L. monocytogenes*, and that lag time was increased further by the inclusion of sodium ascorbate. Other factors, such as moisture content and salt type can influence *L. monocytogenes* growth in sliced deli turkey. The objective of this study was to evaluate how the combination of NaNO<sub>2</sub> concentration, percent pump,

and NaCl or a blend of NaCl/KCl affect the growth of *L. monocytogenes* in vacuum packaged sliced deli turkey stored at 4°C for up to 91 days.

## **Materials and Methods**

### **Experimental Design**

This study consisted of preparing sliced deli turkey formulated with in-going concentrations of 1.5% NaCl or 0.75% NaCl and 0.75% KCl, 0 ppm or 200 ppm NaNO<sub>2</sub>, and using 10% or 45% pump for a total of eight treatments. All treatments were inoculated with *L. monocytogenes* or peptone water (control), vacuum packaged, and stored for 0, 7, 14, 21, 28, 42, 63, or 91 days at 4°C. For each formulation and storage time, three samples of inoculated treatments were evaluated for *L. monocytogenes* populations on MOX and on TAL on days 0, 7, and 14, and on MOX on days 21, 28, 42, 63, and 91. In addition, two samples of control treatments were evaluated for aerobic plate count (APC) at each storage time. The pH, a<sub>w</sub>, residual NaNO<sub>2</sub> concentration, and percent fat, moisture, protein, and sodium was measured using control treatments for each sampling day. The study was replicated three times.

### **Product Preparation**

Eight product formulations (Table 1) were made using water, NaCl (Hi-grade Cargill Salt, J.M. Swank Co., North Liberty, IA), KCl, (Reheis, J.M. Swank Co., North Liberty, IA), cure containing 6.25% NaNO<sub>2</sub> (Blend Tech Cure, J.M. Swank Co., North Liberty, IA), dextrose (ADM Clintose dextrose monohydrate, J.M. Swank Co., North Liberty, IA), sodium phosphate (BK Giulini Brifisol 512, J.M. Swank Co., North Liberty, IA), sodium erythorbate (J.M. Swank Co., North Liberty, IA), and/or carrageenan

(Cargill Aubysel, J.M. Swank Co., North Liberty, IA). Fresh turkey breast meat and pre-ground 1/8" breast meat three days postmortem was obtained from a commercial supplier (Cargill, Springdale, AR). Product temperature was 3.3°C at receipt and turkey was stored overnight in a 4°C cooler before use. Individual brines were made for each treatment first by combining the phosphate with water and then blending the additional ingredients. Whole breasts were injected using a needle injector (Schroder N40, German manufacturer) at 10% or 45% pump depending on the treatment and then macerated (Townsend Storr Macerator PMT-41(S), Netherlands). After maceration the whole breast and pre-ground breast meat were vacuum tumbled (Koch LT 15, Kansas City, MO) for 45 min for the 10% pump treatments or 1 h 30 min for the 45% pump treatments. The meat batter was then stuffed (Handtmann VF 622 Vacuum Filler, West Germany) into a 160 mm plastic casing (Vector SmokeKote 3, Oak Brook, IL) and clipped (Tipper Tie SPR465L, Apex, North Carolina).

| <b>Treatment</b>  | <b>% NaCl</b> | <b>% KCl</b> | <b>% Pump</b> | <b>ppm NaNO<sub>2</sub></b> | <b>Water</b> | <b>Dextrose</b> | <b>Na Erythorbate</b> | <b>PO<sub>4</sub></b> | <b>Carrageenan</b> |
|-------------------|---------------|--------------|---------------|-----------------------------|--------------|-----------------|-----------------------|-----------------------|--------------------|
| <b>S1 M1 N</b>    | 1.50%         | 0.00%        | 10%           | 200                         | 5.45%        | 1.50%           | 0.05%                 | 0.30%                 | 0.00%              |
| <b>S1 M2 N</b>    | 1.50%         | 0.00%        | 45%           | 200                         | 27.0%        | 1.50%           | 0.04%                 | 0.30%                 | 0.45%              |
| <b>S2 M1 N</b>    | 0.75%         | 0.75%        | 10%           | 200                         | 5.45%        | 1.50%           | 0.05%                 | 0.30%                 | 0.00%              |
| <b>S2 M2 N</b>    | 0.75%         | 0.75%        | 45%           | 200                         | 27.0%        | 1.50%           | 0.04%                 | 0.30%                 | 0.45%              |
| <b>S1 M1 No N</b> | 1.50%         | 0.00%        | 10%           | 0                           | 5.79%        | 1.50%           | 0.00%                 | 0.30%                 | 0.00%              |
| <b>S1 M2 No N</b> | 1.50%         | 0.00%        | 45%           | 0                           | 27.3%        | 1.50%           | 0.00%                 | 0.30%                 | 0.45%              |
| <b>S2 M1 No N</b> | 0.75%         | 0.75%        | 10%           | 0                           | 5.79%        | 1.50%           | 0.00%                 | 0.30%                 | 0.00%              |
| <b>S2 M2 No N</b> | 0.75%         | 0.75%        | 45%           | 0                           | 27.3%        | 1.50%           | 0.00%                 | 0.30%                 | 0.45%              |

**Table 1. Ingredient combinations for eight treatments. NaCl - sodium nitrite, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite, Na Erythorbate - sodium erythorbate, PO<sub>4</sub> - phosphate. S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, NoN- 0 ppm NaNO<sub>2</sub>.**

The turkey roasts were thermally processed (Alkar, Model 1000 Oven, Lodi, WI) immediately after formulating following the processing schedule shown in Appendix A and B, cooked to a final internal temperature of 71.7°C and were then placed in a -1.1°C chiller. After four days, the product was hand peeled and then sliced 1.0 - 1.2 mm thick using a deli slicer (Hobart 2621, Troy, OH). The turkey slices were placed three per package into a 20.3 cm x 25.4 cm bag (Sealed Air Cryovac 4700 Series Barrier Bag, Duncan, SC, water transmission rate 0.5-0.6 g/100 in<sup>2</sup>/24 h @ 100°F, 100% relative humidity, oxygen transmission rate 3-6 cm<sup>3</sup>/m<sup>2</sup>/24 h atm @ 40°F, 0% relative humidity) and then the bags were placed into a large Cryovac bag. The large bag was heat sealed using a vacuum packager (Multivac C500, Gepufte Sicherheit, Germany). Products were then stored at 4°C until transported in coolers containing frozen ice packets by car the following day to Kansas State University. The initial meat block was evaluated for *L. monocytogenes* and found negative for all treatments and all three replications.

### ***Listeria monocytogenes* Culture Preparation and Confirmation**

A five-strain cocktail of *L. monocytogenes* (Table 2) was prepared by transferring one mL loopful of each of the five strains into separate tubes containing 9.0 mL TSB (Difco BD, Sparks MD) and incubating at 35°C for 24 ± 2 h. Next, 6.0 mL of each strain was aseptically transferred into a sterile bottle for a total of 30 mL of inoculum, stirred together and used for inoculation of turkey slices.

| <b>Strain</b>                  | <b>Original Source</b>                  |
|--------------------------------|---|
| Mono WPI                       | Wild-type from a meat facility          |
| Mono 38865 P4                  | Unknown Source                          |
| Mono 12243 Serotype 1/2a       | Human isolate from a foodborne outbreak |
| Mono Scot A Serotype 4B        | Human isolate                           |
| Mono CDC H2446 global standard | Bovine                                  |

**Table 2. Strains of *Listeria monocytogenes* used to inoculate deli turkey slices.**

Inoculum level of the 30 mL five-strain *L. monocytogenes* cocktail was initially determined by transferring a one mL aliquot of the cocktail to 9.0 mL 0.1% peptone (Difco BD and Company, Sparks, MD), which was then serially diluted. Dilutions were spread plated in duplicate on MOX (Difco BD and Company, Sparks, MD), incubated at  $35 \pm 2^\circ\text{C}$  for  $48 \pm 2$  h, and counts were reported as log CFU/cm<sup>2</sup>. For confirmation of *L. monocytogenes* cultures, a BD BBL Crystal™ Gram-Positive Identification system (Difco BD and Company, Sparks, MD) was used. This system is a miniaturized identification method employing modified conventional, fluorogenic, and chromogenic substrates intended for the identification of aerobic gram-positive bacteria (Balows and others 1991; Baron and others 1994; Mandell and others 1990; Murray and others 1995). One mL loopful of each of the five strains was transferred to separate tubes of 9.0 mL TSB and incubated at  $35^\circ\text{C}$  for  $24 \pm 2$  h. One mL of each incubated strain was used to hydrate the panel that was then incubated at  $35 \pm 2^\circ\text{C}$  for 18–24 h. The panel lid contained 29 dehydrated substrates and a fluorescence control on tips of plastic prongs and the panel base had 30 reaction wells. The panel was read using a BBL Crystal Panel Viewer which examined for color changes or presence of fluorescence that results from *L. monocytogenes* metabolic activities. The resulting pattern of the 29 reactions was

converted into a ten-digit profile number that was used as the basis for identification in the BBL Crystal ID System (Sneath 1957).

### **Inoculation**

Upon receipt of the treatments at Kansas State University, the master bag was opened. Unsealed vacuum bags containing three turkey deli slices were inoculated with 60  $\mu$ L of *L. monocytogenes* cocktail to have a final inoculation level of approximately 2 log CFU/package. A 10  $\mu$ L spot of *L. monocytogenes* cocktail was micropipetted on each side of the three turkey slices for a total of six spots equaling 60  $\mu$ L. Control packages were similarly inoculated with a total of 60  $\mu$ L of 0.1% peptone water. The packages were then vacuum sealed at  $120 \pm 50$  mm Hg (Multivac A300, Gepufte Sicherheit, Germany) and stored at  $4 \pm 2^{\circ}\text{C}$  for up to 91 days.

### **Media Preparation**

Modified oxford agar (Difco BD and Company, Sparks, MD) was prepared by adding 57.5 g of MOX agar to 1.0 L of distilled water, mixing, and then boiling for 1 min. Next, MOX was transferred to a 1.0 L bottle and sterilized at  $121^{\circ}\text{C}$  for 15 min. Media was then cooled in a stationary waterbath to  $45\text{-}50^{\circ}\text{C}$ . One 10 mL vial of hydrolyzed Oxford antimicrobial supplement (Difco BD and Company, Sparks, MD) was dissolved in 10 mL distilled water and immediately added to the MOX media. This supplement makes the media selective for *L. monocytogenes* (Difco Manual 2009). The media was then poured into 100 x 15 mm disposable agar plates (Difco BD and Company, Sparks, MD) and allowed to set-up for at least 3 h at ambient temperature.

To recover heat-injured *L. monocytogenes*, TAL plates were prepared by adding 40 g of tryptic soy agar (TSA) media to 1.0 L of distilled water, mixing, and then boiling for 1 min or until turbidity was lost. Next, TSA was transferred to a 1.0 L bottle and sterilized at 121°C for 15 min. To an existing solidified MOX plate, 7.0 mL of warm TSA was overlaid by pipetting onto the plate and allowed to set for 20 min. Then, an additional 7.0 mL of TSA was pipetted on the overlay for a total of 14.0 mL TSA overlay (Wu and Fung 2001). The TAL plates were allowed to set-up for an additional 3 h at ambient temperature.

Tryptic soy broth was prepared by adding 30 g of TSB media to 1.0 L of distilled water, mixed thoroughly, and then boiled for 1 min. Then, it was transferred to a 1.0 L bottle and sterilized at 121°C for 15 min.

To prepare peptone for serial dilutions, 1.0 g of Bacto™ Peptone (Difco BD and Company, Sparks, MD) was added to 1.0 L of distilled water, mixed thoroughly, and then sterilized at 121°C for 15 min. Peptone blanks were prepared by dispensing 9.0 mL of 0.1% peptone into 15.0 mL screw-capped tubes and sterilized at 121 °C for 15 min.

### **Enumeration**

For each treatment and storage time, three packages of inoculated treatments were enumerated for *L. monocytogenes* populations and two packages of control treatments were enumerated for APC. This was done by adding 20 mL of 0.1% peptone water diluent to each package and pummeling in a stomacher (Seward Stomacher 400 Laboratory Blender) for 1 min. Serial dilutions were prepared. *Listera monocytogenes* populations were enumerated in duplicate by spread plating 0.1 mL onto MOX and/or TAL plates and incubating at  $35 \pm 2^\circ\text{C}$  for  $48 \pm 2$  h. Aerobic plate count populations were

enumerated in duplicate by plating 1.0 mL onto APC petrifilm (Difco BD and Company, Sparks, MD) and incubating at  $35 \pm 2^\circ\text{C}$  for  $48 \pm 2$  h.

### **Proximate and Chemical Analysis**

Control samples were used to measure  $a_w$ , pH, and moisture, fat, protein, sodium, and residual  $\text{NaNO}_2$  content in duplicate. One package from each treatment was sampled on day 0, 7, 14, 21, 28, 42, 63, and 91 of storage.

To determine pH, a slurry was prepared by combining 10.0 g of sample with 90 mL distilled water in a double-chamber filter bag (Fisherbrand, Ste-Julie, Quebec, Canada), and then stomached for 1 min. The pH was measured with a calibrated pH meter (Acumet AB15, Fischer Scientific, Kent City, Michigan) fitted with a flat-surface combination electrode (13-620-530A; Fisherbrand, Vernon Hills, IL) inserted into the filtered portion of the bag. The pH meter was calibrated using pH 4.0 and pH 7.0 buffer solutions (Fisher Scientific, Fair Lawn, NJ). For each sample, duplicate readings were taken.

Water activity was determined with an  $a_w$  meter (Aqualab CX2 series 3TE; Decagon, Pullman WA) calibrated using a 0.842 NaCl standard (6.0 molal in water; Decagon, Pullman WA) and distilled water (1.0)  $a_w$  at  $25.5^\circ\text{C}$ . Turkey slices were cored using a round meat corer with a diameter of approximately 3.2 cm and placed in a sample container. For each sample, duplicate readings were taken at  $25.5^\circ\text{C}$ .

Fat, moisture, and protein content were determined using AOAC method 2007.04 and a FOSS FoodScan™ Near-Infrared (NIR) Spectrophotometer. Sodium content was determined using AOAC method 969.23. Residual  $\text{NaNO}_2$  concentrations were determined using AOAC method 973.31. For each sample, duplicate readings were taken.

## Statistical Design

A randomized complete block design with sample as the experimental unit was used for microbial data. The treatment structure was a 2 x 2 x 2 factorial with NaNO<sub>2</sub>, salt type, and percent pump. Day was used as a repeated measure. Analysis of variance (ANOVA) was performed using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Least square means were calculated for each independent variable. Statistical significance and tendencies were set at P<0.05.

To determine the effect of storage times on pH, a<sub>w</sub>, residual NaNO<sub>2</sub> concentration, and fat, moisture, protein, and salt content, a one-way classification model using a one-way ANOVA was used. Analysis was performed using the PROC MIXED procedure of SAS. Least square means were calculated for each independent variable. Statistical significance and tendencies were set at P<0.05. The statistical code is presented in Appendix C.

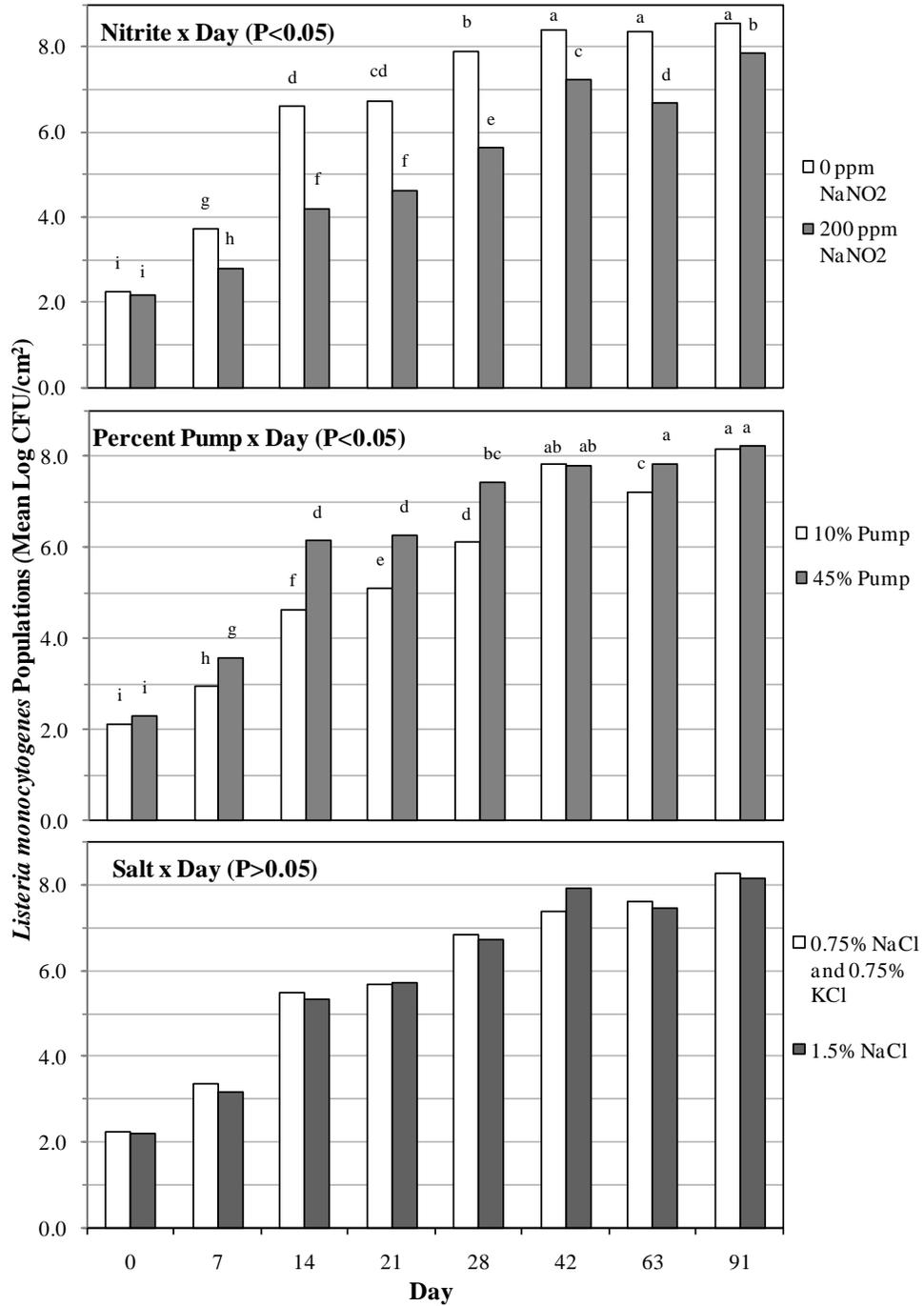
## Results and Discussion

The effects of NaNO<sub>2</sub>, percent pump, and salt type on *L. monocytogenes* growth in sliced deli turkey stored at 4°C are shown in Figure 3-1. There was a NaNO<sub>2</sub> by day interaction (P<0.05) for mean log growth CFU/cm<sup>2</sup> of *L. monocytogenes* on MOX. Initially, *L. monocytogenes* populations averaged 2.23 and 2.19 log CFU/cm<sup>2</sup> for sliced deli turkey formulated with 0 or 200 ppm NaNO<sub>2</sub>, respectively (Table 3). This was close to the target inoculum level of 2 logs CFU/cm<sup>2</sup>. *Listeria monocytogenes* populations in turkey deli slices containing 200 ppm NaNO<sub>2</sub> were 0.70 to 2.39 log CFU/cm<sup>2</sup> lower compared with products formulated with 0 ppm NaNO<sub>2</sub> and stored at 4°C. Sodium nitrite

exerts some inhibitory effect against *L. monocytogenes*, although it does not prevent growth at the concentrations remaining in many cooked meats (Lou and Yousef 1999). In the absence of NaNO<sub>2</sub>, *L. monocytogenes* populations increased to 6.75 log CFU/cm<sup>2</sup> during the first 3 weeks of 4°C storage, while *L. monocytogenes* populations only reached 4.64 log CFU/cm<sup>2</sup> with the inclusion of 200 ppm NaNO<sub>2</sub>. Similarly, Duffy and others (1994) stated that the incorporation of 70-140 mg/kg NaNO<sub>2</sub> significantly reduced the growth of *L. monocytogenes* on vacuum packed cooked meats stored at 5°C.

There was also a percent pump by day interaction (P<0.05) for *L. monocytogenes* populations. Using 10% pump reduced *L. monocytogenes* populations by 0.62 to 1.50 log CFU/cm<sup>2</sup> on days 7 to 28 and at day 63 compared to products pumped to 45%. There are no differences (P>0.05) in *L. monocytogenes* populations by day 91 of storage due to percent pump. According to Bell and Kyriakides (2005), as a<sub>w</sub> approaches, or is lower than, the minimum for growth of *L. monocytogenes*, growth of the organism will be prevented or minimized. In this study, using a 45% pump led to a higher a<sub>w</sub> of 0.975 to 0.979, providing conditions conducive for *L. monocytogenes* growth.

Incorporating 1.5% NaCl or 0.75% NaCl and 0.75% KCl into turkey formulation did not affect (P>0.05) *L. monocytogenes* growth during 91 days of 4°C storage. Bozianis and others (2006) compared the effects of NaCl and KCl on the growth of *L. monocytogenes* and found the effects to be almost identical in terms of growth rates, death rates, lag phase duration, and maximum population.



**Figure 3-1. Effect of nitrite by day, percent pump by day, and salt by day interactions on *Listeria monocytogenes* population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

NaNO<sub>2</sub> - sodium nitrite, NaCl - sodium chloride, KCl - potassium chloride

Means within graphs having different superscripts differ (P<0.05).

**Initial *L. monocytogenes* populations**

| <b>Treatment <sup>a</sup></b> | <b>log CFU/cm<sup>2</sup></b> |
|-------------------------------|-------------------------------|
| <b>S1 M1 N</b>                | 2.30                          |
| <b>S1 M2 N</b>                | 2.15                          |
| <b>S2 M1 N</b>                | 1.91                          |
| <b>S2 M2 N</b>                | 2.40                          |
| <b>S1 M1 No N</b>             | 2.18                          |
| <b>S1 M2 No N</b>             | 2.23                          |
| <b>S2 M1 No N</b>             | 2.12                          |
| <b>S2 M2 No N</b>             | 2.39                          |

No significant difference (P>0.05).

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>a</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl,

M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

**Table 3. Initial *Listeria monocytogenes* populations for all eight treatments on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

There was a percent pump by NaNO<sub>2</sub> interaction (P<0.05) (Table 4). Using a combination of 10% pump and 200 ppm NaNO<sub>2</sub> reduced (P<0.05) *L. monocytogenes* populations by 2.10 log CFU/cm<sup>2</sup> compared to 45% pump and 0 ppm NaNO<sub>2</sub>.

|          | NaNO <sub>2</sub> <sup>a</sup> Concentration |                   |
|----------|--|-------------------|
|          | 0 ppm  | 200 ppm           |
| 10% pump | 6.37 <sup>c</sup>                            | 4.68 <sup>e</sup> |
| 45% pump | 6.78 <sup>b</sup>                            | 5.63 <sup>d</sup> |

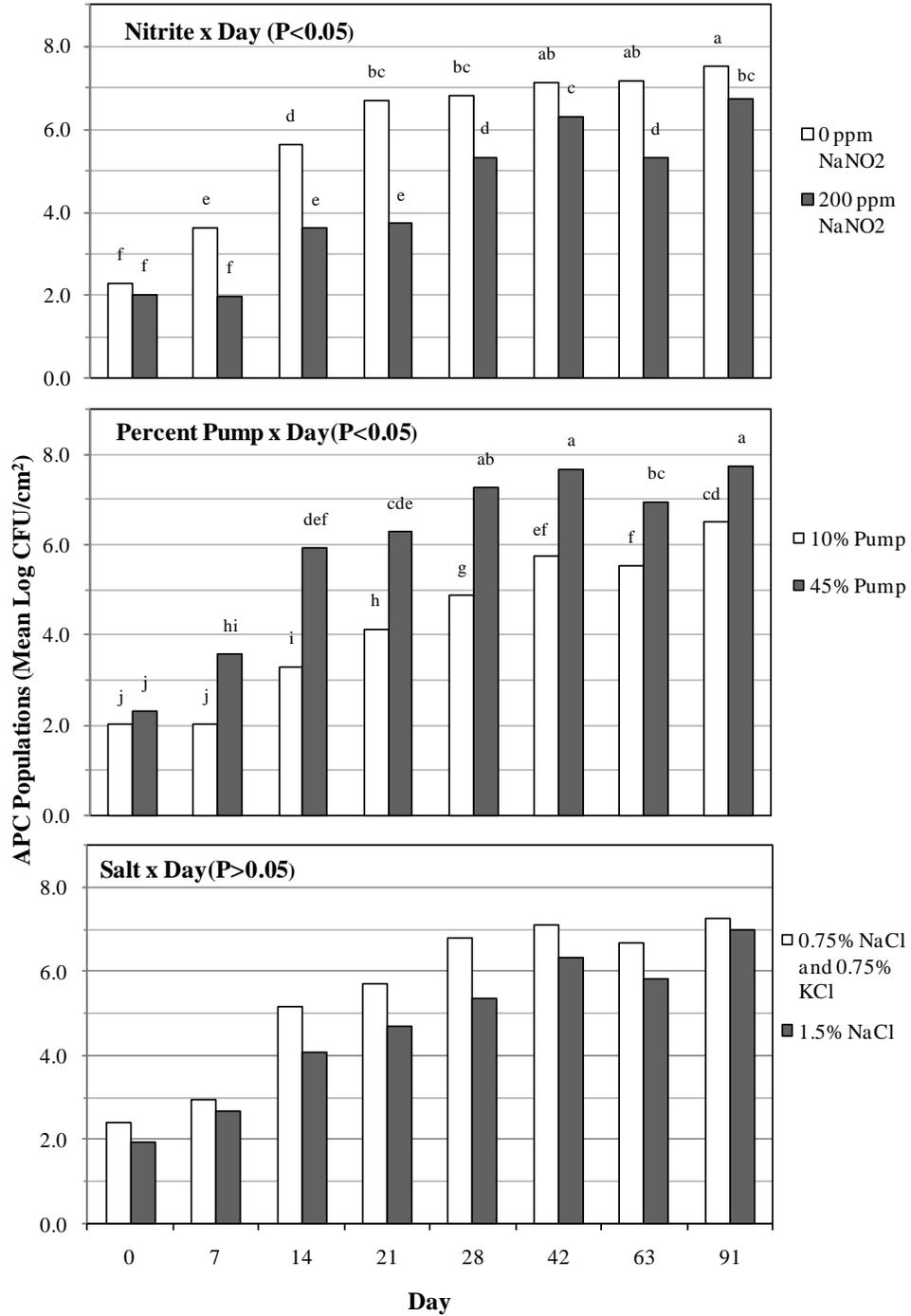
<sup>a</sup> NaNO<sub>2</sub>- sodium nitrite

<sup>b-e</sup> Means with different letters are significantly different (P<0.05).

**Table 4. Interaction of percent pump by nitrite on *Listeria monocytogenes* population means (log CFU/cm<sup>2</sup>) on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

There was a NaNO<sub>2</sub> by day interaction (P<0.05) on mean log APC populations (CFU/cm<sup>2</sup>) in deli turkey slices (Figure 3-2). Aerobic plate count populations were 0.76 to 2.96 log CFU/cm<sup>2</sup> lower on days 7 through 91 of 4°C storage with inclusion of 200 ppm NaNO<sub>2</sub> compared with 0 ppm NaNO<sub>2</sub>. There was also a percent pump by day interaction (P<0.05). Using 10% pump reduced APC populations by 1.25 to 2.64 log CFU/cm<sup>2</sup> during days 7 to 91 compared with 45% pump.

Incorporating 1.5% NaCl or 0.75% NaCl and 0.75% KCl did not affect (P>0.05) APC growth during the 91 days of 4°C storage. Cerruttie and others (2001) found that the effect of a<sub>w</sub> adjusted with NaCl or KCl on the inhibitory action of nisin on *E. coli* cells was not solute-dependent. It seems that apart from the reduced a<sub>w</sub>, there is no added bacteriostatic or bactericidal effect imposed by the different cations of Na<sup>+</sup> or K<sup>+</sup>. Furthermore, the effect of Na<sup>+</sup> or K<sup>+</sup> on the protective effect of nisin against *L. monocytogenes* was the same.

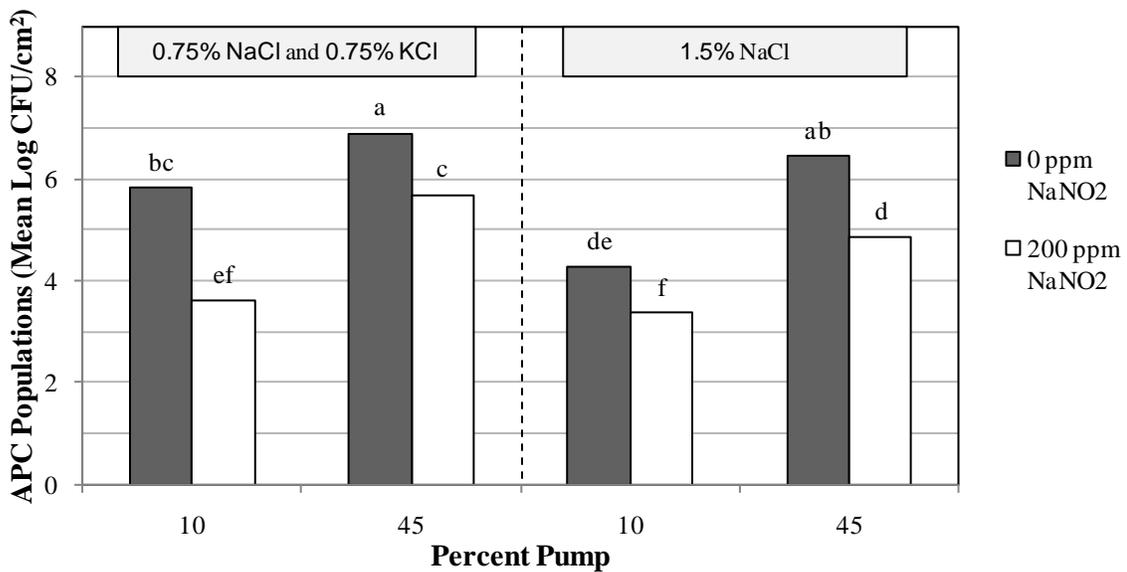


**Figure 3-2. Effect of nitrite by day, percent pump by day, and salt by day interactions on aerobic plate count (APC) population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

NaNO<sub>2</sub> - sodium nitrite, NaCl - sodium chloride, KCl - potassium chloride

Means within graphs having different superscripts differ (P<0.05).

There was a three way interaction ( $P < 0.05$ ) between salt type, percent pump, and  $\text{NaNO}_2$  on mean log APC growth  $\text{CFU}/\text{cm}^2$  (Figure 3-3). Using 10% pump achieved the lowest APC population in sliced deli turkey, within individual salts types and regardless of whether  $\text{NaNO}_2$  was present or absent. The highest APC growth occurred with the combinations of 45% pump and 0 ppm  $\text{NaNO}_2$ , regardless of salt type used. This result is expected as higher percent pump causes a higher  $a_w$  of 0.975 to 0.979, which promotes growth of *L. monocytogenes* and the incorporation of 0 ppm of  $\text{NaNO}_2$ , which inhibits the growth, coupled together would give the most optimum combination for the highest APC growth.



**Figure 3-3. Effect of salt by percent pump by nitrite interaction on aerobic plate count (APC) population means in vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

**$\text{NaNO}_2$  - sodium nitrite, NaCl - sodium chloride, KCl - potassium chloride**

**Means with different superscripts differ ( $P < 0.05$ ).**

Enumerating *L. monocytogenes* on MOX or TAL media did not affect ( $P>0.05$ ) *L. monocytogenes* populations (Table 5). However, Wu and Fung (2003) found a higher ( $P<0.05$ ) recovery of heat-injured cells by approximately 1 log when TAL was used in comparison to MOX. There were no heat-injured cells in this study since *L. monocytogenes* inoculation took place after the turkey roast was thermally processed, cooled, and sliced. Since *L. monocytogenes* counts on MOX and TAL were similar up to day 14, the decision was made to only use MOX media for the remainder of the storage day evaluations.

| Treatment <sup>a</sup> | Media Type |         |
|------------------------|------------|---------|
|                        | MOX        | TAL     |
| S1 M1 N                | 2.67 **    | 2.63 ** |
| S1 M2 N                | 3.25 *     | 3.27 *  |
| S2 M1 N                | 2.64 **    | 2.90 *  |
| S2 M2 N                | 3.67 *     | 3.73 *  |
| S1 M1 NoN              | 3.76 *     | 3.81 *  |
| S1 M2 NoN              | 4.49 **    | 4.60 *  |
| S2 M1 NoN              | 3.88 *     | 3.99 *  |
| S2 M2 NoN              | 4.59 *     | 4.70 *  |

No significant difference ( $P>0.05$ ).

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>a</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl,

M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

Standard Error = \* 0.20, \*\* 0.21

**Table 5. Comparison of *Listeria monocytogenes* population means on eight treatments of vacuum packaged sliced deli turkey during 4°C storage for up to 91 days using Modified Oxford Media (MOX) or Thin Agar Layer (TAL).**

Data for pH,  $a_w$ , proximate analysis, and sodium content are presented in Table 6. The pH and fat content were similar for all treatments and averaged 6.2 and 0.67%, respectively. According to Glass and Doyle (1989) *L. monocytogenes* generally grows well on meats near or above pH 6.0 and poorly or not at all on products near or below pH 5.0. Water activity was 0.966 to 0.979 for all treatments and was lower ( $P < 0.05$ ) for treatments with 10% pump than 45% pump. The optimum  $a_w$  for the growth of *L. monocytogenes* is approximately 0.97 (Bell and Kyriakides 2005), and all treatments were near the optimum  $a_w$  for growth. Moisture content in treatments with 10% pump was similar ( $P > 0.05$ ) with a mean 73.5% moisture, however, slight differences ( $P < 0.05$ ) occurred in moisture content among treatments with 45% pump which ranged from 77.5% to 78.7%. Percent protein in treatments with 45% pump was similar ( $P > 0.05$ ) with a mean of 15.3% protein, however, there were differences ( $P < 0.05$ ) in percent protein among treatments with 10% pump, ranging from 17.9% to 19.2%. As expected, as moisture content increased, percent protein decreased. Percent sodium was different ( $P < 0.05$ ) among all treatments and ranged from 1.78% to 2.56%. The range across all treatments could be due to the variation of sodium content in the incoming raw materials, however, this was not measured.

| <b>Treatment</b> <sup>1</sup> | <b>pH</b> | <b>a<sub>w</sub></b> <sup>2</sup> | <b>% Moisture</b>   | <b>% Fat</b> | <b>% Protein</b>    | <b>% Sodium</b>     |
|-------------------------------|-----------|-----------------------------------|---------------------|--------------|---------------------|---------------------|
| <b>S1 M1 N</b>                | 6.22      | 0.966 <sup>d</sup>                | 73.53 <sup>c</sup>  | 0.60         | 18.48 <sup>bc</sup> | 2.56 <sup>a</sup>   |
| <b>S1 M2 N</b>                | 6.16      | 0.975 <sup>b</sup>                | 77.48 <sup>b</sup>  | 0.67         | 15.44 <sup>d</sup>  | 2.09 <sup>bc</sup>  |
| <b>S2 M1 N</b>                | 6.28      | 0.969 <sup>c</sup>                | 73.51 <sup>c</sup>  | 0.74         | 18.84 <sup>ab</sup> | 2.21 <sup>b</sup>   |
| <b>S2 M2 N</b>                | 6.15      | 0.978 <sup>ab</sup>               | 78.27 <sup>ab</sup> | 0.58         | 15.24 <sup>d</sup>  | 1.93 <sup>cd</sup>  |
| <b>S1 M1 No N</b>             | 6.28      | 0.964 <sup>d</sup>                | 73.2 <sup>c</sup>   | 0.74         | 17.96 <sup>c</sup>  | 2.75 <sup>a</sup>   |
| <b>S1 M2 No N</b>             | 6.13      | 0.978 <sup>ab</sup>               | 78.67 <sup>a</sup>  | 0.61         | 15.18 <sup>d</sup>  | 1.94 <sup>cd</sup>  |
| <b>S2 M1 No N</b>             | 6.19      | 0.978 <sup>c</sup>                | 73.76 <sup>c</sup>  | 0.73         | 19.24 <sup>a</sup>  | 2.02 <sup>bcd</sup> |
| <b>S2 M2 No N</b>             | 6.17      | 0.979 <sup>a</sup>                | 78.55 <sup>ab</sup> | 0.69         | 15.46 <sup>d</sup>  | 1.78 <sup>d</sup>   |
| <b>SE</b> <sup>3</sup>        | 0.10      | 0.00                              | 0.27                | 0.11         | 0.32                | 0.14                |

<sup>a-d</sup> Means within columns having different superscripts differ (P<0.05).

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>1</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

<sup>2</sup> a<sub>w</sub> = water activity.

<sup>3</sup> Standard error.

**Table 6. Proximate and chemical analysis by treatment interaction means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

There was a pH by day, percent fat content by day, and sodium by day interaction ( $P < 0.05$ ) (Table 7). Although pH was similar ( $P > 0.05$ ) on days 0 to 42, pH decreased ( $P < 0.05$ ) on days 63 and 91. Glass and Doyle (1989) stated that in vacuum packaged sliced turkey, the pH decreased from 6.46 to 5.32 after six weeks at 4.4°C storage, probably due to the fermentation of available carbohydrate by lactic acid bacteria. The fat content was similar ( $P > 0.05$ ) on days 0 to 21, but was lower ( $P < 0.05$ ) on days 28 to 91. The sodium content ranged from 1.94 to 2.41 and tended to be lower during the first 21 days of storage.

| <b>Day</b> | <b>pH</b>         | <b>% Fat</b>      | <b>% Sodium</b>    |
|------------|-------------------|-------------------|--------------------|
| <b>0</b>   | 6.36 <sup>a</sup> | 0.82 <sup>a</sup> | 2.08 <sup>bc</sup> |
| <b>7</b>   | 6.34 <sup>a</sup> | 0.85 <sup>a</sup> | 1.94 <sup>c</sup>  |
| <b>14</b>  | 6.37 <sup>a</sup> | 0.85 <sup>a</sup> | 2.06 <sup>bc</sup> |
| <b>21</b>  | 6.30 <sup>a</sup> | 0.94 <sup>a</sup> | 1.97 <sup>c</sup>  |
| <b>28</b>  | 6.27 <sup>a</sup> | 0.44 <sup>b</sup> | 2.29 <sup>ab</sup> |
| <b>42</b>  | 6.25 <sup>a</sup> | 0.52 <sup>b</sup> | 2.32 <sup>a</sup>  |
| <b>63</b>  | 5.98 <sup>b</sup> | 0.42 <sup>b</sup> | 2.41 <sup>a</sup>  |
| <b>91</b>  | 5.72 <sup>c</sup> | 0.51 <sup>b</sup> | 2.23 <sup>ab</sup> |

<sup>a-c</sup> Means within columns having different superscripts differ ( $P < 0.05$ ).

**Table 7. Proximate and chemical analysis by day interaction means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

Changes in residual  $\text{NaNO}_2$  for all treatments during storage are shown in (Figure 3-4. There was a treatment by day interaction ( $P < 0.05$ ) for residual  $\text{NaNO}_2$ . Residual  $\text{NaNO}_2$  was similar ( $P > 0.05$ ) within each sample day for all the treatments containing 0 ppm  $\text{NaNO}_2$ . Residual  $\text{NaNO}_2$  was also similar ( $P > 0.05$ ) across all shelf life days of storage within a treatment for sliced deli turkey containing 0 ppm  $\text{NaNO}_2$ . Residual  $\text{NaNO}_2$  was similar ( $P > 0.05$ ) within treatments containing 200 ppm  $\text{NaNO}_2$  and 10% pump on sample days 0 and 14. Treatments formulated with 10% pump and 200 ppm  $\text{NaNO}_2$  tended to have less residual  $\text{NaNO}_2$  compared treatments formulated with 45% pump and 200 ppm  $\text{NaNO}_2$ . Overall, treatments formulated with 200 ppm  $\text{NaNO}_2$  showed a trend to decrease in residual  $\text{NaNO}_2$  with the exception of a few spikes. In general, residual  $\text{NaNO}_2$  decreased in turkey deli slices during storage. In a study by Honikel (2008), the largest decrease of  $\text{NaNO}_2$  was observed during the manufacturing process through the end of the heating process. This early loss amounts up to about 65% of the  $\text{NaNO}_2$  independent of the ingoing concentrations. Within 20 days of cold storage,  $\text{NaNO}_2$  concentrations drop further to a third of the concentration after heating. In this study, no major spikes occurred after day 21 and there was a continual decrease in residual  $\text{NaNO}_2$  during storage. The numerical data for residual  $\text{NaNO}_2$  during storage is presented in Appendix D.

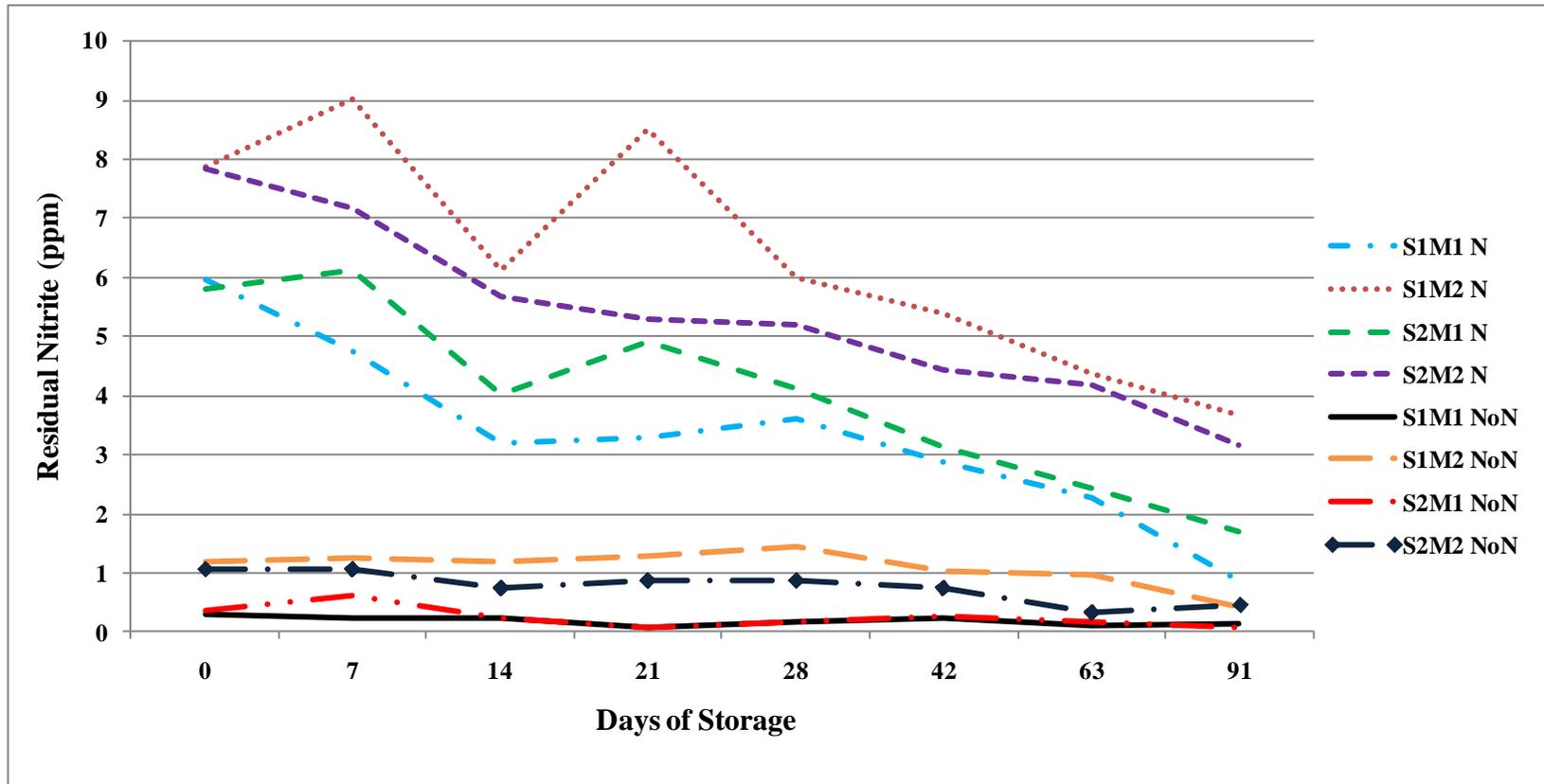


Figure 3-4. Residual nitrite (ppm) means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days. NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite. Eight treatments of sliced deli turkey: S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

Changes in *L. monocytogenes* populations for all treatments during storage are shown in Figure 3-5. There was a treatment by day interaction ( $P < 0.05$ ) for *L. monocytogenes* populations. The initial inoculum level averaged 2.21 log CFU/cm<sup>2</sup> and was similar ( $P > 0.05$ ) for all treatments on day 0. *Listeria monocytogenes* populations increased ( $P < 0.05$ ) from day 0 to 14 by 1.30 to 5.04 log CFU/cm<sup>2</sup>. Treatments containing 200 ppm NaNO<sub>2</sub> and 10% pump had less ( $P < 0.05$ ) *L. monocytogenes* growth than treatments with 200 ppm NaNO<sub>2</sub> and 45% pump on days 14 to 28. Overall, *L. monocytogenes* populations increased during storage and by 91 days of storage *L. monocytogenes* populations were similar regardless of level of NaNO<sub>2</sub> used except for treatments formulated with 0.75% NaCl/0.75% KCl and 10% pump. Sodium nitrite concentration and percent pump played an important role in *L. monocytogenes* growth. Sodium nitrite concentration and percent pump were the intrinsic factors that influenced *L. monocytogenes* growth. The numerical data for *L. monocytogenes* populations during storage is presented in Appendix E.

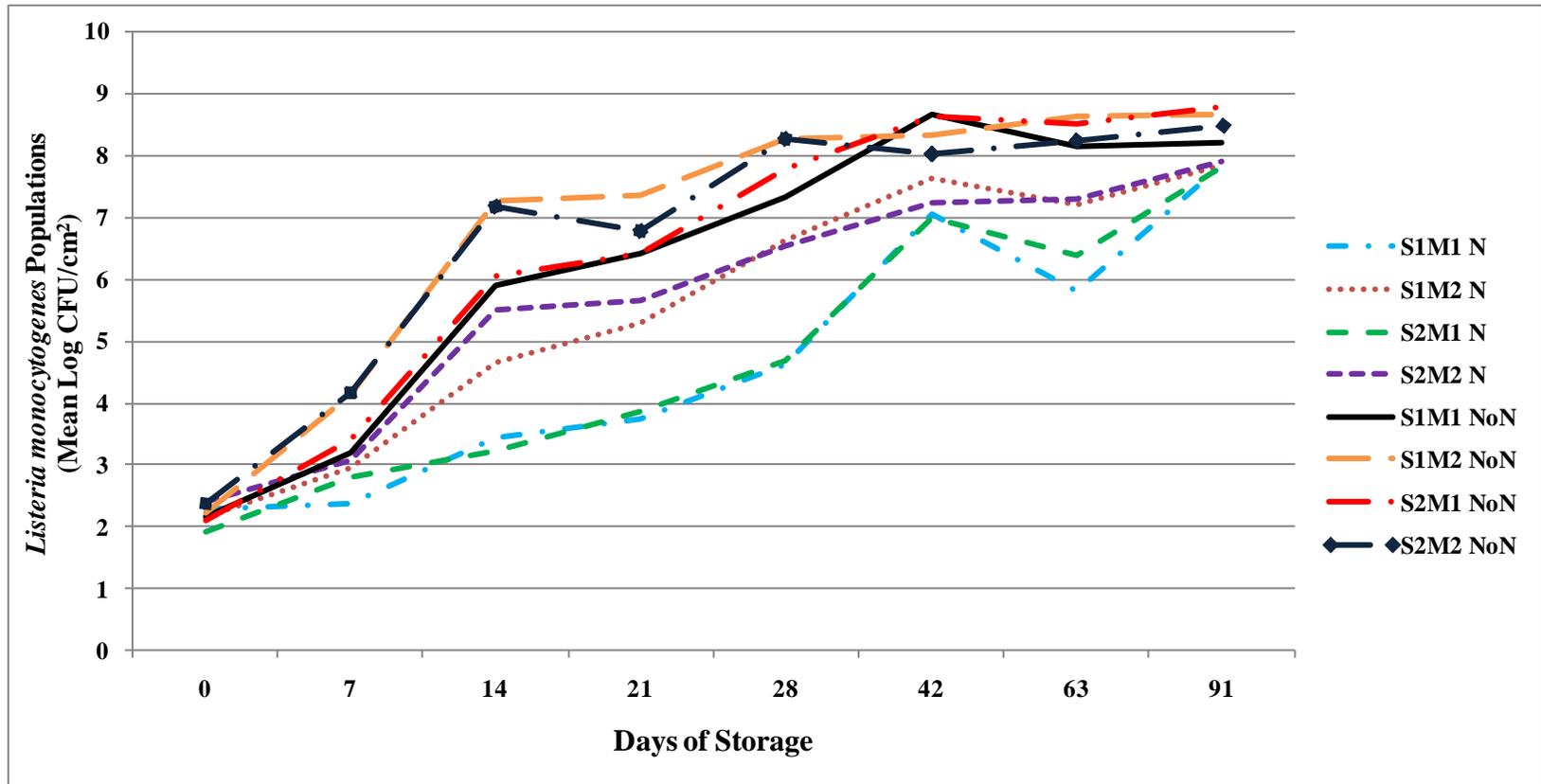


Figure 3-5. *Listeria monocytogenes* population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days. NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite. Eight treatments of sliced deli turkey: S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

Changes in APC populations for all treatments during storage are shown in Figure 3-6. There was a treatment by day interaction ( $P < 0.05$ ) for APC populations. The initial APC population averaged  $2.16 \log \text{CFU/cm}^2$  and was similar ( $P > 0.05$ ) for all treatments on day 0. On days 7 to 21, treatments containing 0 ppm  $\text{NaNO}_2$  and 45% pump had similar APC ( $P > 0.05$ ) populations and were higher ( $P < 0.05$ ) than treatments with 0 ppm  $\text{NaNO}_2$  and 10% pump. By day 14 of storage treatments formulated with 45% pump and 0 ppm  $\text{NaNO}_2$  were at the index of spoilage with APC populations of approximately  $7 \log \text{CFU/cm}^2$ . By day 28, the 45% pump, 200 ppm  $\text{NaNO}_2$ , and 0.75%  $\text{NaCl}/0.75\% \text{KCl}$  treatment reached spoilage, while the 45% pump, 200 ppm  $\text{NaNO}_2$ , and 1.5%  $\text{NaCl}$  treatment did not reach spoilage until day 42. All treatments showed an increasing rate of APC growth across all shelf life days. The numerical data for APC populations during storage is presented in Appendix F.

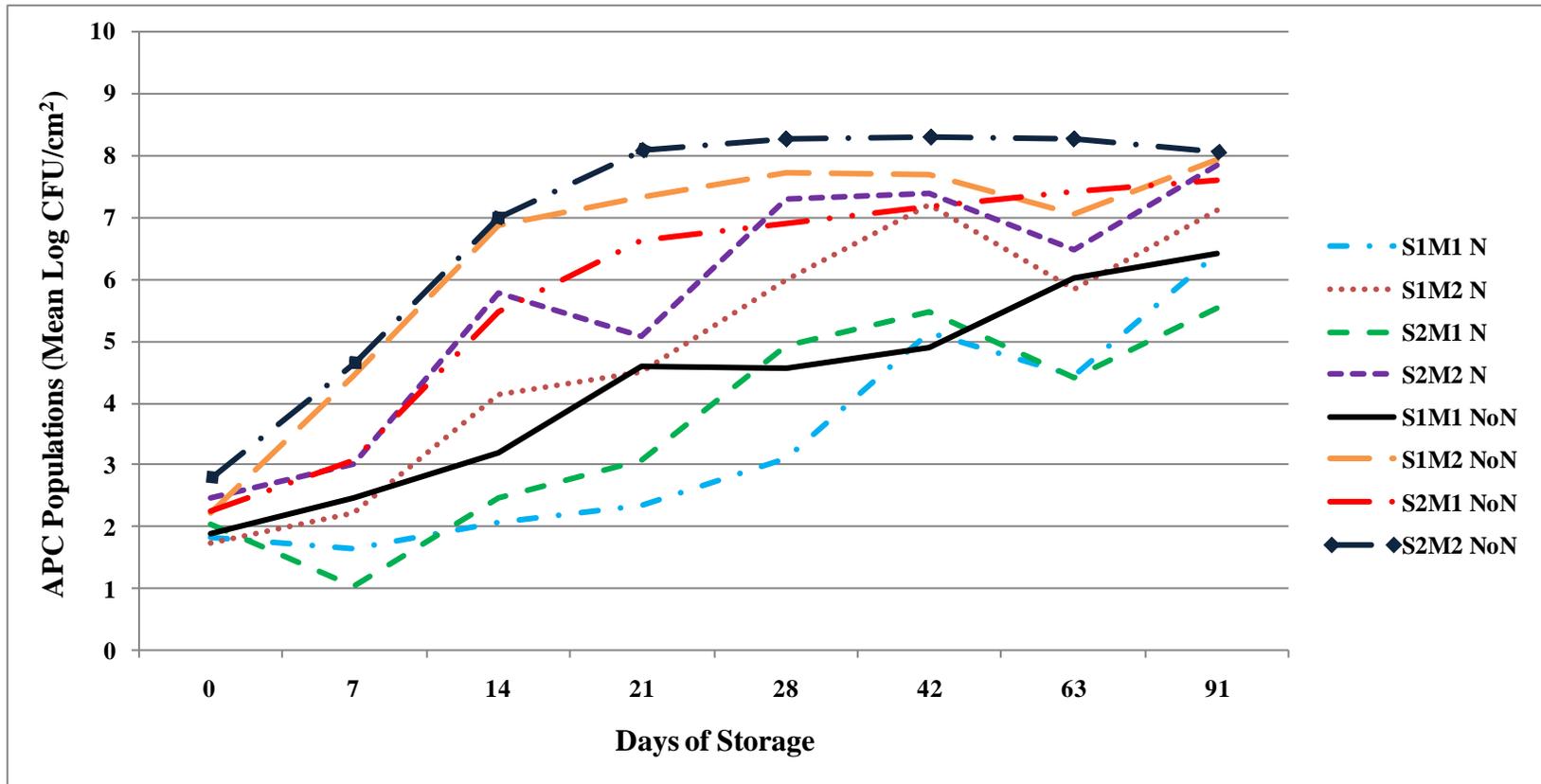


Figure 3-6. Aerobic plate count (APC) populations on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days. NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite. Eight treatments of sliced deli turkey: S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, No N- 0 ppm NaNO<sub>2</sub>.

## Chapter 4 - Conclusions

The effect of intrinsic factors plays a major role in the ability of *L. monocytogenes* to grow and survive in vacuum packaged sliced deli turkey during refrigerated storage. *Listeria monocytogenes* populations in sliced deli turkey were affected by the interaction of NaNO<sub>2</sub> and percent pump, while there was no effect of NaCl or the blend of NaCl and KCl. The influence of these factors on the growth of *L. monocytogenes* is due to the hurdle effect, which is widely accepted as an illustration for the complex interaction of several inhibitory factors in the preservation of foods (Leistner 1978). Results of this study indicated that *L. monocytogenes* populations would be lower when turkey deli meat is formulated using 200 ppm NaNO<sub>2</sub>, compared to using 0 ppm NaNO<sub>2</sub>. Also, the use of 45% percent pump provided more moisture and promoted higher populations of *L. monocytogenes* compared to a 10% percent pump. Finally, using 1.5% NaCl or the combination of 0.75% NaCl/0.75% KCl showed no affect on *L. monocytogenes* populations. Sodium nitrite had an inhibitory effect on *L. monocytogenes* populations even in the presence of different percent pumps and using NaCl or a combination of NaCl and KCl. The percent pump had an effect on *L. monocytogenes* populations in conjunction with NaNO<sub>2</sub> and NaCl or the blend of NaCl and KCl.

It is important to understand how the interaction of intrinsic factors influence *L. monocytogenes* growth. This can lead to improved product formulation and safe wholesome products for the consumer.

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## Appendix A - Turkey Deli Roast Cook Cycle

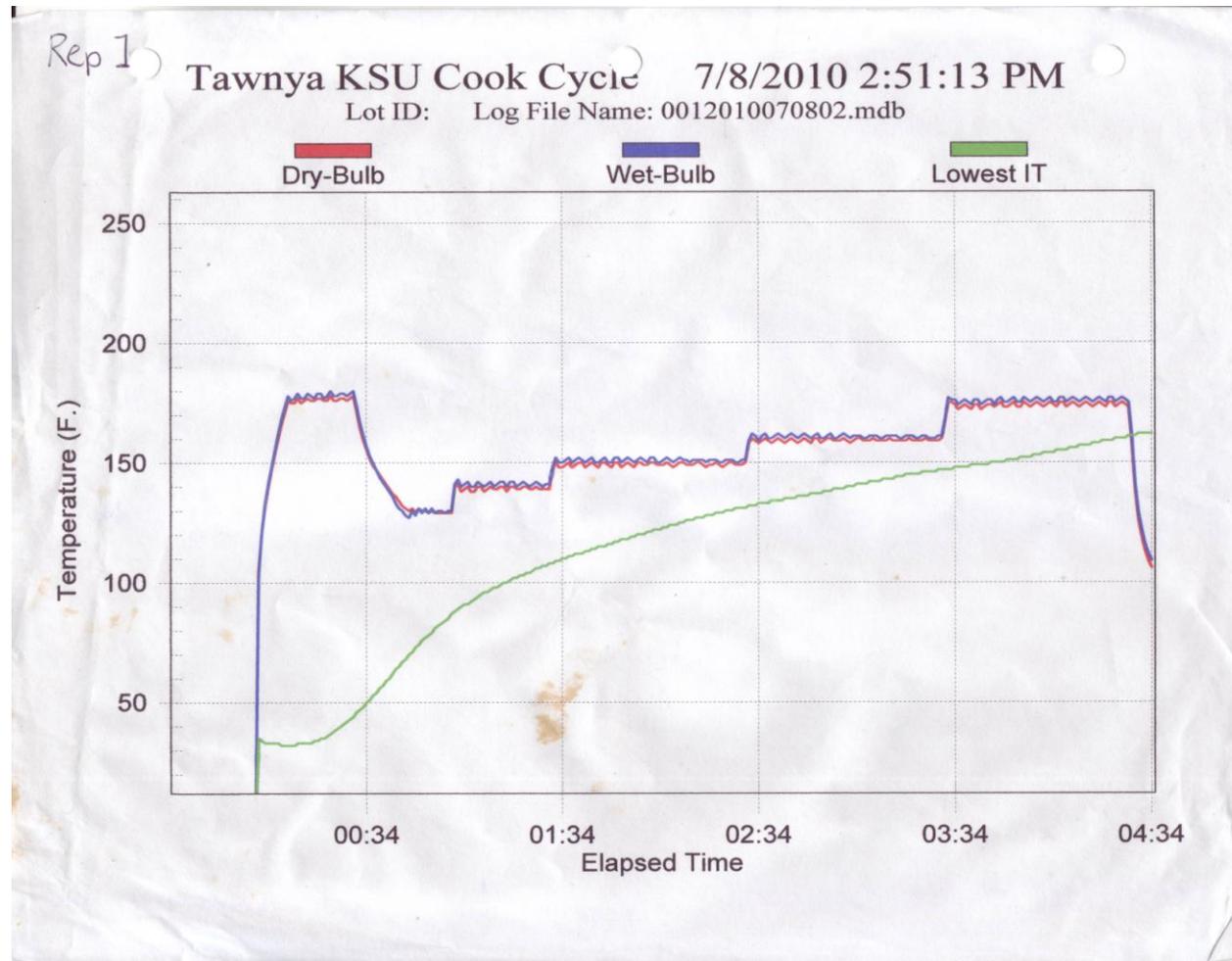


Figure 4-1. Cooking cycle used for the thermal processing of the deli sliced turkey.

## Appendix B - Alkar Cook Program for Deli Turkey

| Alkar Cook Program for Deli Turkey Roast <sup>1</sup> |             |           |       |       |      |       |      |             |             |          |         |    | Shower |              | Smoke Generation |       | Liquid Smoke |  | Idle After |
|---|-------------|-----------|-------|-------|------|-------|------|-------------|-------------|----------|---------|----|--------|--------------|------------------|-------|--------------|--|------------|
| Step #  | Step Type   | Step Time | DB °C | WB °C | rH   | IT °C | pH   | Main Blower | Exhaust Fan | Humidity | Dampers | On | Off    | Preheat time | On               | Dwell | Step         |  |            |
| 1   | Steam Cook  | 0:30      | 82.2  | 82.2  | 100% | --    | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 2   | Steam Cook  | 0:30      | 54.5  | 54.5  | 100% | --    | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 3   | Steam Cook  | 0:30      | 60.0  | 60.0  | 100% | --    | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 4   | Steam Cook  | 1:00      | 65.4  | 65.4  | 100% | --    | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 5   | Steam Cook  | 1:00      | 71.1  | 71.1  | 100% | --    | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 6   | Steam Cook  | 0:01      | 79.4  | 79.4  | 100% | 71.1  | 0.00 | 10          | Off         | Off      | Closed  | -- | --     | --           | --               | --    | Off          |  |            |
| 7   | Cold Shower | 0:30      | 10.0  | 0.0   | 100% | --    | 0.00 | 0           | On          | Off      | Auto    | -- | --     | --           | --               | --    | Off          |  |            |

<sup>1</sup> DB = Dry bulb, WB = Wet bulb, rH = Relative Humidity, IT = Internal temperature

**Figure 4-2. Cook program print out of cooking cycle for the deli turkey.**

## Appendix C - Statistical Codes

### Statistical code in SAS to run all proximate and chemical analysis.

```
data;  
input trt rep samp day nitrite;  
datalines;  
Enter data here  
  
;  
proc mixed;  
class trt rep samp day;  
model nitrite = trt|day;  
random rep samp;  
lsmeans trt|day;  
lsmeans day/pdiff;  
lsmeans trt/pdiff;  
lsmeans trt*day/pdiff;  
run;  
quit;
```

Replace nitrite with the other proximate and chemical analysis and their data.

### Statistical code in SAS to run all *L. monocytogenes* on MOX and APC

```
options nocenter;  
ata;  
input Rep Trt Sample Bag_Number Salt  
Moisture Nitrite Inoc Day MOX_Log  
APC pH aw Fat Protein Moisture_2 Salt_2  
Nitrite_conc;  
datalines;  
  
Enter data here  
  
proc mixed;  
class sample rep trt salt moisture nitrite day;  
model mox_log = salt|moisture|nitrite|day;  
random rep sample(rep trt);  
lsmeans nitrite*day moisture*day moisture*nitrite /pdiff;  
run;  
quit;
```

## Appendix D - Treatment by Day Interactions for Residual Sodium Nitrite

| Treatment <sup>1</sup> | Days of Storage    |                     |                     |                     |                      |                       |                      |                     | SE   |
|------------------------|--------------------|---------------------|---------------------|---------------------|----------------------|-----------------------|----------------------|---------------------|------|
|                        | 0                  | 7                   | 14                  | 21                  | 28                   | 42                    | 63                   | 91                  |      |
| <b>S1 M1 N</b>         | 5.97 <sup>bv</sup> | 4.76 <sup>cvw</sup> | 3.19 <sup>bx</sup>  | 3.29 <sup>cx</sup>  | 3.62 <sup>cwx</sup>  | 2.87 <sup>cx</sup>    | 2.26 <sup>bcxy</sup> | 0.86 <sup>bcy</sup> | 0.53 |
| <b>S1 M2 N</b>         | 7.87 <sup>av</sup> | 9.02 <sup>av</sup>  | 6.12 <sup>aw</sup>  | 8.51 <sup>av</sup>  | 6.00 <sup>aw</sup>   | 5.41 <sup>awx</sup>   | 4.36 <sup>axy</sup>  | 3.68 <sup>ay</sup>  | 0.53 |
| <b>S2 M1 N</b>         | 5.81 <sup>bv</sup> | 6.12 <sup>bcv</sup> | 4.03 <sup>bwx</sup> | 4.92 <sup>bvw</sup> | 4.12 <sup>bcwx</sup> | 3.12 <sup>bcxy</sup>  | 2.43 <sup>byz</sup>  | 1.69 <sup>bz</sup>  | 0.53 |
| <b>S2 M2 N</b>         | 7.85 <sup>av</sup> | 7.19 <sup>bv</sup>  | 5.69 <sup>aw</sup>  | 5.29 <sup>bwx</sup> | 5.21 <sup>abwx</sup> | 4.45 <sup>abwxy</sup> | 4.18 <sup>axy</sup>  | 3.15 <sup>ay</sup>  | 0.53 |
| <b>S1 M1 NoN</b>       | 0.29 <sup>cv</sup> | 0.22 <sup>dv</sup>  | 0.23 <sup>cv</sup>  | 0.07 <sup>dv</sup>  | 0.17 <sup>dv</sup>   | 0.25 <sup>dv</sup>    | 0.12 <sup>dv</sup>   | 0.13 <sup>cv</sup>  | 0.53 |
| <b>S1 M2 NoN</b>       | 1.20 <sup>cv</sup> | 1.26 <sup>dv</sup>  | 1.18 <sup>cv</sup>  | 1.28 <sup>dv</sup>  | 1.44 <sup>dv</sup>   | 1.02 <sup>dv</sup>    | 0.98 <sup>cdv</sup>  | 0.42 <sup>bcv</sup> | 0.53 |
| <b>S2 M1 NoN</b>       | 0.35 <sup>cv</sup> | 0.61 <sup>dv</sup>  | 0.24 <sup>cv</sup>  | 0.06 <sup>dv</sup>  | 0.18 <sup>dv</sup>   | 0.25 <sup>dv</sup>    | 0.17 <sup>dv</sup>   | 0.08 <sup>cv</sup>  | 0.53 |
| <b>S2 M2 NoN</b>       | 1.07 <sup>cv</sup> | 1.05 <sup>dv</sup>  | 0.75 <sup>cv</sup>  | 0.85 <sup>dv</sup>  | 0.87 <sup>dv</sup>   | 0.74 <sup>dv</sup>    | 0.35 <sup>dv</sup>   | 0.45 <sup>bcv</sup> | 0.53 |

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>1</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, NoN- 0 ppm NaNO<sub>2</sub>.

<sup>a-d</sup> Least square means within columns having different superscripts differ (P<0.05).

<sup>v-z</sup> Least square means within rows having different superscripts differ (P<0.05).

**Table 8. Treatment by day interaction of residual sodium nitrite (ppm) means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

## Appendix E - Treatment by Day Interactions for *Listeria monocytogenes*

| Treatment <sup>1</sup> | Days of Storage       |                       |                       |                                      |                        |                            |                        |                        |
|------------------------|-----------------------|-----------------------|-----------------------|--------------------------------------|------------------------|----------------------------|------------------------|------------------------|
|                        | 0                     | 7                     | 14                    | 21                                   | 28                     | 42                         | 63                     | 91                     |
| <b>S1 M1 N</b>         | 2.30 <sup>ay *</sup>  | 2.39 <sup>cy *</sup>  | 3.43 <sup>dx **</sup> | 3.75 <sup>dw<sub>x</sub> ***</sup>   | 4.62 <sup>dw *</sup>   | 7.07 <sup>cdu ****</sup>   | 5.81 <sup>dv **</sup>  | 7.83 <sup>bcu **</sup> |
| <b>S1 M2 N</b>         | 2.15 <sup>ax *</sup>  | 2.95 <sup>bcx *</sup> | 4.65 <sup>cw *</sup>  | 5.30 <sup>cw ****</sup>              | 6.63 <sup>cv *</sup>   | 7.62 <sup>bcdu ****</sup>  | 7.21 <sup>bcuv *</sup> | 7.86 <sup>bcu *</sup>  |
| <b>S2 M1 N</b>         | 1.91 <sup>az **</sup> | 2.79 <sup>bcy *</sup> | 3.24 <sup>dxy *</sup> | 3.86 <sup>dx *</sup>                 | 4.70 <sup>dw *</sup>   | 7.00 <sup>duv ****</sup>   | 6.39 <sup>cdv *</sup>  | 7.81 <sup>cu *</sup>   |
| <b>S2 M2 N</b>         | 2.40 <sup>ay *</sup>  | 3.09 <sup>bcy *</sup> | 5.52 <sup>bx *</sup>  | 5.66 <sup>bcw<sub>x</sub> ****</sup> | 6.54 <sup>cvw *</sup>  | 7.23 <sup>cduv ****</sup>  | 7.30 <sup>buv *</sup>  | 7.89 <sup>bcu *</sup>  |
| <b>S1 M1 NoN</b>       | 2.18 <sup>az *</sup>  | 3.19 <sup>bcy *</sup> | 5.90 <sup>bx *</sup>  | 6.41 <sup>abw<sub>x</sub> ****</sup> | 7.33 <sup>bcvw *</sup> | 8.65 <sup>au ****</sup>    | 8.14 <sup>auv *</sup>  | 8.22 <sup>abcu *</sup> |
| <b>S1 M2 NoN</b>       | 2.23 <sup>ay *</sup>  | 4.13 <sup>ax *</sup>  | 7.27 <sup>aw **</sup> | 7.37 <sup>avw ****</sup>             | 8.28 <sup>auv *</sup>  | 8.34 <sup>abuv ****</sup>  | 8.64 <sup>au *</sup>   | 8.66 <sup>abu *</sup>  |
| <b>S2 M1 NoN</b>       | 2.12 <sup>ay *</sup>  | 3.42 <sup>abx *</sup> | 6.05 <sup>bw *</sup>  | 6.42 <sup>abw ****</sup>             | 7.78 <sup>abv *</sup>  | 8.65 <sup>auv ****</sup>   | 8.52 <sup>auv *</sup>  | 8.80 <sup>au *</sup>   |
| <b>S2 M2 NoN</b>       | 2.39 <sup>ay *</sup>  | 4.19 <sup>ax *</sup>  | 7.18 <sup>avw *</sup> | 6.78 <sup>aw ****</sup>              | 8.27 <sup>au *</sup>   | 8.05 <sup>abcuv ****</sup> | 8.24 <sup>au *</sup>   | 8.51 <sup>abcu *</sup> |

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>1</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, NoN- 0 ppm NaNO<sub>2</sub>.

<sup>a-d</sup> Least square means within columns having different superscripts differ (P<0.05).

<sup>u-z</sup> Least square means within rows having different superscripts differ (P<0.05).

\* SE = 0.49, \* SE = 0.50, \*\*\* SE = 0.52, \*\*\*\* SE = 0.53

**Table 9. Treatment by day interaction of *Listeria monocytogenes* population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

## Appendix F - Treatment by Day Interactions for Aerobic Plate Count

| Treatment <sup>1</sup> | Days of Storage       |                                    |                                  |  |  |                                    |                                    |                                   |
|------------------------|-----------------------|------------------------------------|----------------------------------|--|--|------------------------------------|------------------------------------|-----------------------------------|
|                        | 0                     | 7                                  | 14                               | 21   | 28   | 42                                 | 63                                 | 91                                |
| S1 M1 N                | 1.84 <sup>ayz</sup> * | 1.65 <sup>cdz</sup> *              | 2.07 <sup>dyz</sup> *            | 2.34 <sup>fyz</sup> *                          | 3.10 <sup>exy</sup> *                        | 5.14 <sup>bv<sub>w</sub></sup> *   | 4.43 <sup>dw<sub>x</sub></sup> **  | 6.41 <sup>bc<sub>v</sub></sup> ** |
| S1 M2 N                | 1.75 <sup>ay</sup> *  | 2.23 <sup>bcd<sub>y</sub></sup> *  | 4.13 <sup>cx</sup> *             | 4.49 <sup>dew<sub>x</sub></sup> ****           | 5.99 <sup>bc<sub>v<sub>w</sub></sub></sup> * | 7.20 <sup>av</sup> ***             | 5.85 <sup>cv<sub>w</sub></sup> **  | 7.12 <sup>ab<sub>v</sub></sup> *  |
| S2 M1 N                | 2.05 <sup>axy</sup> * | 1.04 <sup>dy</sup> *               | 2.47 <sup>dx</sup> *             | 3.09 <sup>efw<sub>x</sub></sup> **             | 4.93 <sup>cd<sub>v</sub></sup> *             | 5.48 <sup>b<sub>v</sub></sup> *    | 4.42 <sup>dw<sub>x</sub></sup> *   | 5.53 <sup>cv</sup> **             |
| S2 M2 N                | 2.47 <sup>ay</sup> *  | 3.00 <sup>b<sub>y</sub></sup> *    | 5.77 <sup>b<sub>x</sub></sup> *  | 5.09 <sup>cd<sub>x</sub></sup> ***             | 7.31 <sup>av<sub>w</sub></sup> *             | 7.39 <sup>av<sub>w</sub></sup> **  | 6.47 <sup>bcw<sub>x</sub></sup> ** | 7.86 <sup>av</sup> *              |
| S1 M1 NoN              | 1.89 <sup>az</sup> *  | 2.48 <sup>bc<sub>yz</sub></sup> ** | 3.18 <sup>cd<sub>y</sub></sup> * | 4.58 <sup>dx</sup> *                           | 4.56 <sup>dx</sup> *                         | 4.91 <sup>bw<sub>x</sub></sup> *** | 6.03 <sup>cv<sub>w</sub></sup> **  | 6.42 <sup>bc<sub>v</sub></sup> *  |
| S1 M2 NoN              | 2.22 <sup>ax</sup> *  | 4.45 <sup>aw</sup> *               | 6.86 <sup>av</sup> *             | 7.34 <sup>ab<sub>v</sub></sup> ***             | 7.72 <sup>av</sup> *                         | 7.71 <sup>av</sup> ***             | 7.06 <sup>abc<sub>v</sub></sup> *  | 7.95 <sup>av</sup> *              |
| S2 M1 NoN              | 2.26 <sup>ax</sup> *  | 3.07 <sup>b<sub>x</sub></sup> *    | 5.47 <sup>b<sub>w</sub></sup> *  | 6.63 <sup>bc<sub>v<sub>w</sub></sub></sup> *** | 6.91 <sup>ab<sub>v</sub></sup> *             | 7.19 <sup>av</sup> *               | 7.41 <sup>ab<sub>v</sub></sup> *   | 7.59 <sup>ab<sub>v</sub></sup> *  |
| S2 M2 NoN              | 2.83 <sup>ay</sup> *  | 4.65 <sup>ax</sup> *               | 6.98 <sup>aw</sup> *             | 8.07 <sup>av<sub>w</sub></sup> *               | 8.27 <sup>av<sub>w</sub></sup> *             | 8.30 <sup>av</sup> *               | 8.27 <sup>av</sup> *               | 8.06 <sup>av<sub>w</sub></sup> *  |

NaCl - sodium chloride, KCl - potassium chloride, NaNO<sub>2</sub> - sodium nitrite

<sup>1</sup> S1- 1.5% NaCl, S2- 0.75% NaCl and 0.75% KCl, M1- 10% pump, M2- 45% pump, N- 200 ppm NaNO<sub>2</sub>, NoN- 0 ppm NaNO<sub>2</sub>.

<sup>a-f</sup> Least square means within columns having different superscripts differ (P<0.05).

<sup>v-z</sup> Least square means within rows having different superscripts differ (P<0.05).

\* SE = 0.61, \*\* SE = 0.64, \*\*\* SE = 0.69, \*\*\*\* SE = 0.76

**Table 10. Treatment by day interaction of aerobic plate count (APC) population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days.**

**Appendix G - Numerical Data for Nitrite, Percent Pump, and  
Salt Type by Day for *Listeria monocytogenes***

|                           | Day  |      |      |      |      |      |      |      |
|---------------------------|------|------|------|------|------|------|------|------|
|                           | 0    | 7    | 14   | 21   | 28   | 42   | 63   | 91   |
| <b>Nitrite</b>            |      |      |      |      |      |      |      |      |
| 0 ppm NaNO <sub>2</sub>   | 2.25 | 3.73 | 6.60 | 6.75 | 7.92 | 8.42 | 8.38 | 8.55 |
| 200 ppm NaNO <sub>2</sub> | 2.19 | 2.80 | 4.21 | 4.64 | 5.62 | 7.23 | 6.68 | 7.85 |
| <b>Percent Pump</b>       |      |      |      |      |      |      |      |      |
| 10% Pump                  | 2.14 | 2.95 | 4.65 | 5.11 | 6.11 | 7.84 | 7.22 | 8.16 |
| 45% Pump                  | 2.30 | 3.59 | 6.15 | 6.28 | 7.43 | 7.81 | 7.84 | 8.23 |
| <b>Salt</b>               |      |      |      |      |      |      |      |      |
| 0.75% NaCl and 0.75% KCl  | 2.22 | 3.37 | 5.50 | 5.68 | 6.82 | 7.37 | 7.61 | 8.25 |
| 1.5% NaCl                 | 2.22 | 3.17 | 5.31 | 5.71 | 6.72 | 7.92 | 7.45 | 8.14 |

**Figure 4-3. Interaction of nitrite, percent pump, and salt by day on *Listeria monocytogenes* population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days. NaNO<sub>2</sub> - sodium nitrite, NaCl - sodium chloride, KCl - potassium chloride.**

**Appendix H - Numerical Data for Nitrite, Percent Pump, and  
Salt Type by Day for Aerobic Plate Count**

|                           | Day  |      |      |      |      |      |      |      |
|---------------------------|------|------|------|------|------|------|------|------|
|                           | 0    | 7    | 14   | 21   | 28   | 42   | 63   | 91   |
| <b>Nitrite</b>            |      |      |      |      |      |      |      |      |
| 0 ppm NaNO <sub>2</sub>   | 2.30 | 3.62 | 5.63 | 6.69 | 6.80 | 7.13 | 7.17 | 7.50 |
| 200 ppm NaNO <sub>2</sub> | 2.03 | 1.98 | 3.61 | 3.73 | 5.33 | 6.31 | 5.31 | 6.74 |
| <b>Percent Pump</b>       |      |      |      |      |      |      |      |      |
| 10% Pump                  | 2.01 | 2.02 | 3.30 | 4.13 | 4.88 | 5.76 | 5.54 | 6.50 |
| 45% Pump                  | 2.32 | 3.58 | 5.94 | 6.29 | 7.26 | 7.68 | 6.95 | 7.75 |
| <b>Salt</b>               |      |      |      |      |      |      |      |      |
| 0.75% NaCl and 0.75% KCl  | 2.40 | 2.94 | 5.17 | 5.71 | 6.80 | 7.10 | 6.66 | 7.27 |
| 1.5% NaCl                 | 1.93 | 2.66 | 4.06 | 4.71 | 5.34 | 6.34 | 5.83 | 6.98 |

**Figure 4-4. Interaction of nitrite, percent pump, and salt by day on aerobic plate count (APC) population means on vacuum packaged sliced deli turkey during 4°C storage for up to 91 days. NaNO<sub>2</sub> - sodium nitrite, NaCl - sodium chloride, KCl - potassium chloride.**