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Control of *Listeria monocytogenes* in Ready-to-Eat Meat Containing Levulinate, Lactate, or Lactate and Diacetate

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CONTROL OF *LISTERIA MONOCYTOGENES* IN READY-TO-EAT MEAT CONTAINING LEVULINATE, LACTATE, OR LACTATE AND DIACETATE

by

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ABSTRACT

Control of *Listeria monocytogenes* in Ready-to-Eat Meat Containing Levulinate, Lacate, or Lactate and Diacetate

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Control of the pathogen *Listeria monocytogenes* in ready-to-eat (RTE) meats is a major concern in the food industry. The objective of this study was to compare the growth of *L. monocytogenes* on refrigerated RTE meats containing sodium levulinate (4-oxopentanoic acid, a five carbon organic acid with GRAS status), sodium lactate, or a combination of sodium lactate and sodium diacetate. Turkey roll and bologna were prepared to contain (wt/wt) sodium lactate (2%); sodium lactate in combination with sodium diacetate (1.875% sodium lactate, 0.125% sodium diacetate); sodium levulinate (1, 2, or 3%); or no antilisterial additive. Samples were sliced, inoculated with a 5-strain cocktail (10^2 to 10^3 CFU/cm^2) of *L. monocytogenes*, vacuum packaged, and stored at 2°C for 0-12 weeks.

Triplicate packages of each treatment were analyzed bi-weekly for growth of the pathogen. Bacterial counts exceeded 10^5 CFU/cm^2 in controls after 4 weeks in turkey and over 10^6 CFU/cm^2 after 8 weeks in bologna. In turkey, *L. monocytogenes* showed significant growth in samples containing sodium lactate after 6 weeks (>10^4 CFU/cm^2) and after 8 weeks when used in combination with diacetate. Further, samples containing 1% sodium levulinate did not show significant growth of the pathogen for 10 weeks (~10^4 CFU/cm^2), while those containing 2% and 3% levulinate inhibited growth for 12 weeks. In bologna, adding any antimicrobial inhibited growth for 12 weeks.

Finally, *Listeria*–free samples of turkey roll and bologna, containing the various organic acid salts, were evaluated by members of consumer taste panels. Statistical analysis (ANOVA) showed that there were no differences in overall liking of samples of turkey roll ($p = 0.19$) or bologna ($p = 0.42$). In turkey, sodium levulinate was more effective at preventing growth of *L. monocytogenes*, while in bologna it was as effective as the current industry standards lactate and diacetate. Addition of levulinate did not alter the sensory acceptability of either product.

(69 pages)

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INTRODUCTION

Listeria monocytogenes is a gram-positive, aerobic or facultatively aerobic, non-sporeforming, rod-shaped bacterium. *L. monocytogenes* resists the deleterious effects of freezing, drying and heating remarkably well for a non-sporeformer (35). *L. monocytogenes* grows in a variety of different environments ranging from soil, plants, and water to intracellular mammalian tissues (35). Several species of *Listeria* are pathogenic to ruminants and other animals, and *L. monocytogenes* is a major human pathogen of food-borne origin (43). Listeriosis, the disease caused by *L. monocytogenes,* is characterized by a high mortality rate often greater than 20% for immunosuppressed individuals, pregnant women, fetuses and neonates (8). When live bacteria are ingested, *Listeria* colonizes the intestine and moves from the intestine to the bloodstream. From there it can infect the inner organs, the brain, the central nervous system, and the fetus in pregnant women because it is capable of crossing the intestinal membrane, as well as the blood-brain and feto-placental barriers (8).

Systemic infection by *L. monocytogenes*, termed listeriosis, is not characterized by a unique set of symptoms. The most common symptoms are meningitis and sepsis. The overall health of the host greatly determines the seriousness of the disease. Healthy people are *not* very susceptible to listeriosis; however, only a small number of cells is necessary to infect immunocompromised people such as those with HIV infection, alcoholism, and cancer. Other groups of people that are highly susceptible to listeriosis include diabetics (especially Type I) and pregnant women.

The U.S. Center for Disease Control (CDC) has estimated that about 2,500 cases of listeriosis occur each year, 99% of which are transmitted through food (31). Efforts to control food-borne transmission of *L. monocytogenes* are problematic because the pathogen is ubiquitous and commonly found in and on food, such as fresh meat and poultry (17). Ready-to-eat (RTE) meats are of particular concern if the pathogen contaminates products after thermal processing. Therefore, it is necessary to develop methods to prevent or inhibit the growth of *L. monocytogenes* during storage of RTE foods in order to improve the safety of the products (3).

Recent listeriosis outbreaks associated with ready-to-eat meat and poultry products (10, 11, 12) prompted the US Department of Agriculture-Food Safety and Inspection Service (USDA-FSIS) to issue a rule encouraging the addition of antimicrobial agents to control *L. monocytogenes* in RTE meat products (10, 11, 12). Specifically, establishments are required to include measures in their hazard analysis critical control point (HACCP) plans that prevent proliferation of *Listeria* on RTE meat products that support the growth of *L. monocytogenes* and that are exposed to the environment after cooking (18).

Various salts of lactic, acetic and other organic acids have demonstrated antimicrobial activity toward *L. monocytogenes* in RTE foods when used at levels between 1.5 and 3% (3, 4, 5, 7, 24, 32, 33, 44, 45, 46, 47, 50, 51, 59, 60). These antimicrobials do not necessarily kill microorganisms, but rather inhibit their growth by causing an extended lag phase. As a result of such findings, one and a half to three percent lactate is currently added to RTE meats to control the growth of *L.*

monocytogenes. Tompkin (56) states that the most widely used additives include sodium lactate and sodium diacetate, used individually or in combination.

It is important for the meat industry to continue to expand its supply of anti-listerial ingredients because new combinations of antimicrobials will increase the effectiveness of *L. monocytogenes* control measures and rotation of different anions may help prevent the emergence of resistant strains. There has recently been a renewed interest in the anti-listerial activity of various generally recognized as safe (GRAS) organic acids and their salts because their commercial application is simple and cost-effective compared to other control strategies such as high heat, high hydrostatic pressure, or irradiation. Levulinic acid (4-oxopentanoic acid) is a commercially available 5-carbon organic acid that has GRAS status for direct addition to food as a flavoring agent or adjunct (21 CFR, 172.515). Levulinic acid is a by-product of corn extrusion and may have antimicrobial activity similar to other organic acids (57). Levulinic acid's molecular formula is $C_5H_8O_3$, with a molecular weight of 116.12, and a pKa of 4.59. In previous research, it was shown that 1.4% sodium levulinate inhibited bacterial growth in fresh sausage as effectively as a higher level (2.7%) of sodium lactate (57).

Hypothesis

Our hypothesis is that sodium levulinate is an effective antilisterial agent when added to ready-to-eat meat products (bologna and turkey roll) and that the sodium levulinate does not negatively impact the organoleptic characteristics of these products.

Objectives

Objective 1: Evaluate the extent to which levulinate inhibits the growth of *Listeria monocytogenes* in ready-to-eat meat products as compared to industry standards of lactate and acetate.

Objective 2: Establish the impact of sodium levulinate on the sensory acceptability of turkey roll and bologna as compared to lactate and a mixture of lactate and diacetate.

LITERATURE REVIEW

Listeria

Listeria monocytogenes was first described by Murray (34), who named it *Bacterium monocytogenes* because of an increase in the number of circulating monocytes (i.e., monocytosis) found in infected laboratory guinea pigs and rabbits. It was renamed *Listeria hepatolytica* by Pirie in 1927, and was given its present name, *Listeria monocytogenes,* in 1940 (16). The first case confirmed by isolation of the bacterium from an infected human was made in 1929 by Nyfeldt (16).

L. monocytogenes is a gram-positive, non-sporeforming, facultatively anaerobic rod that is capable of growth between -0.4 and 50°C (16). It is oxidase negative and catalase positive. *L. monocytogenes* is ubiquitous in the environment and able to withstand various environmental and processing stresses such as high salt concentrations, low pH level, and refrigeration temperatures (3). Within a narrow temperature range, *L. monocytogenes* possesses peritrichous flagella, which give it a tumbling motility. When grown between 20 and 25°C, flagella are produced and assembled at the cell surface, but at higher temperatures (e.g., 37°C) flagellin production is notably reduced (16). At optimum temperatures for growth, the bacterium is able to grow at lower pH levels, while at less than optimum temperatures the pH levels need to be more neutral for growth to occur. At 4°C the minimum pH required for growth is between pH 5.0 to 5.7, and at 30°C the minimum pH is between 4.3 and 5.2 (2).

While pasteurization and cooking methods used by processors kill *Listeria monocytogenes*, post-processing contamination may occur because the organism is so

prevalent in the environment. A series of outbreaks during the 1980s greatly increased interest in the disease, especially among food manufacturers and governmental agencies (16). Post-processing contamination of RTE foods is a serious concern and has led to several recent *Listeria* outbreaks.

One of the largest meat recalls in history occurred in October 2002, when 27.4 million pounds of fresh and frozen ready-to-eat turkey and chicken products were recalled after a multi-state listeriosis outbreak. Eight states reported a total of 53 cases, resulting in eight deaths and three stillbirths or miscarriages (12). In late 1998, over 50 illnesses were linked to a rare strain of *L. monocytogenes* that was found in a large frankfurter and deli meat plant (9). Six deaths and two miscarriages resulted in the ten states affected. From May to November in 2000, ten states reported 29 cases of listeriosis from deli turkey meat, resulting in four deaths and three stillbirths or miscarriages (11).

In response to these and many other outbreaks, the Food Safety and Inspection Service of the US Department of Agriculture issued a regulation that requires establishments that produce ready-to-eat meat or poultry products to have measures in their HACCP plans to prevent contamination of the product with *Listeria monocytogenes.* These measures should prevent proliferation of *Listeria* on RTE meat products that support the growth of *L. monocytogenes* and especially those products that are exposed to the environment after cooking (18). These rules offer manufacturers three options which determine the frequency and stringency of regulatory testing. The first includes implementing *both* a post-process lethality step and an antimicrobial additive to control outgrowth. The second requires the use of *either* a post-process lethality step *or* the

addition of an antimicrobial. The third solely implements the use of appropriate sanitation. The first alternative qualifies for the lowest testing frequency; the second, moderate testing frequency; and the third alternative requires the highest testing frequency.

Verification measures used by the FSIS to determine the effectiveness of antimicrobial agents used as ingredients in RTE products are based on the suppression of listerial growth during refrigerated shelf life of the products. Agents that allow greater than 2 log growth are generally not eligible as antimicrobials, while those that allow less than 1 log growth are accepted additives for the control of *Listeria monocytogenes.* Products that contain accepted additives as a means of inhibiting listeria qualify for less stringent testing (18).

As a result, the meat industry now includes various salts of lactic, acetic and other organic acids at levels between 1.5 and 3% in their processed meat products to inhibit *L. monocytogenes.* Tompkin (56) states that the most widely used compounds include sodium lactate and sodium diacetate, used individually or in combination. These acids do not necessarily kill microorganisms, but rather inhibit their growth by causing an extended lag phase. Although lactate and diacetate are effective inhibitors of *L. monocytogenes* on cured products, they have been shown to be less effective on non cured RTE products. Therefore, it is critical for alternative antimicrobials to be identified that provide better inhibition of *Listeria monocytogenes* on cured as well as on uncured RTE meat products (23).

Listeriosis

Human systemic infection with *L. monocytogenes* is termed listeriosis. Listeriosis is characterized by a high mortality rate of over 20% in infected individuals. Although listeriosis accounts for less than 2% of food borne illness in the United States, it has been responsible for 40% of the deaths caused by a food borne illness (10). The U.S. Center for Disease Control (CDC) has estimated that about 2,500 cases of listeriosis occur each year, 99% of which are transmitted through food (31).

Immunosuppressed individuals, pregnant women, fetuses and neonates are most susceptible to *Listeria* infections (8). The infective dose of *L. monocytogenes* is currently unknown, although it appears to be above 100 viable cells depending on pathogen strain and susceptibility of the host (38). When sufficient numbers of live bacteria are ingested, *Listeria* will colonize the gastrointestinal tract, then move from the intestine to the bloodstream, where it spreads to the organs, the brain, and the fetus of pregnant women. *Listeria* is able to infect these organs because it is capable of crossing the intestinal membrane, the blood-brain barrier, and the feto-placental barriers (8).

There are many virulence factors in *L. monocytogenes* that contribute to its ability to cross biological barriers. One such factor is a major surface protein internalin (InlA) which has been shown to play a prominent role in entry of *L. monocytogenes* into cultured epithelial cells, a key step in *L. monocytogenes* pathogenicity (22, 48). Other factors that contribute to virulence include proteins listeriolysin O (LLO) and PlcA, which promote escape from the phagocytic vacuole by hemolytic activity and the production of phospholipases, respectively; proteins ActA and PlcB, which are necessary for intracellular actin-based motility and cell-to-cell spread; and Hyl, a pore-forming

cytolysin (48). The genes encoding these proteins, with the exception of *inlA*, are clustered on a 10-kb virulence locus consisting of three transcriptional units, and are all regulated by PrfA, a transcriptional activator encoded by the *prfA* gene (22, 48).

L. monocytogenes readily spreads from cell to cell after invading a host (37). Once the bacterium has attached to a host cell, it is taken up into a primary vacuole by inducing its own endocytosis. *L. monocytogenes* can then escape the vacuole into the host cytoplasm by production of LLO, a pore-forming hemolytic protein, and PlcA, as discussed above (37). In the cytoplasm, the bacterium utilizes host proteins to attain intracellular actin-based motility, promoting its intercellular spread from the cytoplasm of one infected cell into the cytoplasm of another (37).

Listeriosis is not characterized by a unique set of symptoms, but the most common symptoms are meningitis and sepsis of tissues and blood. The CDC describes meningitis as an infection of the fluid of a person's spinal cord and the fluid that surrounds the brain (13). High fever, headache, and stiff neck are common symptoms. Other symptoms may include nausea, vomiting, discomfort looking into bright lights, confusion, and sleepiness. In newborns and small infants the classic symptoms of fever, headache, and neck stiffness may be absent. The infant may only appear slow or inactive, or be irritable, have vomiting, or be feeding poorly. As the disease progresses, there arises a high risk of seizures.

Sepsis is a severe illness caused by overwhelming infection of the bloodstream, usually by toxin-producing bacteria. The symptoms can be similar to meningitis and include fever or hypothermia, hyperventilation, chills, shaking, skin rash, rapid heartbeat, confusion or delirium and a decreased urine output (6).

Although healthy people are *not* very susceptible to listeriosis, only a small number of cells is necessary to infect immunocompromised people, including those with HIV infection, alcoholics, and cancer patients. Diabetics (especially Type I) and pregnant women are also very susceptible to listeriosis. In perinatal infection, pregnant women will usually contract a mild influenza-like illness, and rarely exhibit a full-blown case of listeriosis (16). However, the disease is often passed to the fetus. Symptoms of neonatal listeriosis include respiratory distress syndrome, rash, purulent conjunctivitis, pneumonia, hyperexcitablity, vomiting, cramps, and fever (16).

Weak Acid Inhibition of Bacterial Growth

Various salts of lactic, acetic and other organic acids demonstrate antimicrobial activity toward *L. monocytogenes* in RTE foods (3, 4, 32, 33, 44, 47). Accordingly, one and a half to three percent acid salts (wt/wt formulations) are added to processed meats to control *Listeria*. The most widely used compounds include sodium lactate and sodium diacetate, used individually or in combination (56).

Organic acid preservatives (such as lactate and acetate) inhibit microbial spoilage of foods and beverages by extending the lag phase, rather than by killing the microbe. Despite the widespread use of weak organic acids, the mechanisms responsible for microbe inhibition are largely unknown. There are three putative mechanisms by which acid anions inhibit bacterial growth: acidification of the cytoplasm (14,19, 26, 28, 36, 53, 55); accumulation of anions in the cytoplasm (40, 41, 42); and disruption of the lipid membrane (19, 25, 54).

It is clear that undissociated (protonated) acid molecules diffuse into the cytoplasm through the cell membrane (28, 36, 53). Because the pH is near neutrality in the cytoplasm, the weak acid dissociates into anions and protons. The anions cannot diffuse across the plasma membrane so they accumulate in the cell. The proton is pumped back across the membrane at an energy cost to the cell. This proton pumping will consequently cause an increase in protonated acid species, and this species will enter the cell at a higher frequency. As the protonated species enters more frequently, it is dissociated and higher concentrations of both the proton and anion accumulate in the cell. If the rate of proton accumulation exceeds that of efflux systems, the internal pH begins to fall and the cytoplasm becomes more acidic. This acidification of the cytoplasm may repress growth by inhibiting glycolysis (14, 26), preventing active transport (19), or by interfering with signal transduction (55).

Increasing evidence suggests that accumulation of anions within the cell may be more inhibitory to growth than release of the proton, especially in a mildly acidic environment as is found in most RTE meat products (40, 41, 42). Anion accumulation may inhibit various metabolic functions of the cell. Specifically, accumulation of acetate anion caused intracellular methionine depletion and the accumulation of homocysteine, a toxic methionine intermediate in *E. coli*. The inhibitory action of this acid could be assuaged by addition of methionine to the growth medium (40). Other studies have shown that when *E.coli* is grown in the presence of 8 mM acetic acid in a minimal medium, with the external pH buffered to 6.0, the rate of growth is inhibited by 50% and the internal concentration of the acetate anion reaches a steady state of approximately 250 mM (39). Such a high concentration of anion significantly increases the osmolarity of

the cytoplasm, causing potentially lethal increases in turgor pressure as the flow of water into the cell increases. However, the cell compensates for this by reducing the concentrations of other solutes in the cytoplasm. In particular, glutamate, which is the largest single pool of osmotically active anions (constituting 25% of the total anion pool in some cells). In *E. coli*, the glutamate pools were reduced by about 80% when grown in 8 mM acetate with a pH of 6.0 (39).

Another prospective mechanism for microorganism inhibition is the perturbation of membrane function by the undissociated weak acids (19, 54). Undissociated weak acids are known to partition into membrane lipid bilayers at concentrations that are dependant upon their lipophilicity (i.e., their ability to dissolve in fats, oils, lipids, and non-polar solvents). There is a good correlation between the inhibition by the weak acids and their respective lipophilicities. Stratford and Anslow (54) demonstrated that in yeast cells the minimum inhibitory concentration of sorbic acid was much lower than that of acetic acid, which correlated with the much greater lipid solubility of sorbic acid. In theory, small organic molecules interfere with membrane proteins by inhibiting the transport of substrate molecules into cells, leading to growth inhibition (25).

In summary, the preservation of foods by the addition of weak acids may be due to the concerted effects of various possible mechanisms. The available evidence indicates that weak acids inhibit microorganisms by lowering the internal pH of the cells, interrupting membrane functions, and interfering with the osmotic properties and metabolic functions resulting from the accumulation of anions in the cytoplasm.

Levulinic Acid

Because it is important for the meat industry to continue to expand its supply of antilisterial ingredients, there has recently been a renewed interest in the antilisterial activity of various GRAS organic acids and their salts. These compounds are attractive because their commercial application is simple and cost-effective compared to other control strategies (i.e., high heat, high hydrostatic pressure or irradiation).

Levulinic acid, or 4-oxopentanoic acid, is a commercially available 5-carbon organic acid that has GRAS status for direct addition to food as a flavoring agent or adjunct (21 CFR, 172.515). Its molecular formula is $C_5H_8O_3$, with molecular weight of 116.12, and a pKa of 4.59.

Levulinic acid can be produced by high temperature acid hydrolysis of carbohydrates, such as glucose, galactose, sucrose, fructose, chitose, and biometric material such as wood, starch and agricultural wastes (21). A new method for producing levulinic acid from biomass has significantly improved the cost effectiveness of supplying this chemical to industries. This new method can produce levulinic acid for only $0.04 to $0.10 per pound (27).

Sodium levulinate has not been extensively studied as an antimicrobial agent in meat products. Thus, there are currently no USDA regulations for use of sodium levulinate in RTE meat products. However, previous research has shown that 1.4% sodium levulinate inhibited growth of spoilage bacteria in fresh pork and turkey sausages as effectively as a higher level (2.7%) of sodium lactate (57). Because the work done by Vasavada et al. (57) showed that sodium levulinate was very effective at inhibiting

spoilage bacteria on fresh meat products, this study investigated the anti-listerial action of sodium levulinate in RTE turkey and bologna products.

MATERIALS AND METHODS

Bacterial Strains

Five strains of *Listeria monocytogenes*, including J1-177 (serotype 1/2b, human isolate), C1-056 (serotype 1/2a, human isolate), N3-013 (serotype 4b, food isolate), R2-499 (serotype 1/2a, sliced turkey isolate), N1-227 (serotype 4b, food isolate) were used in this study. The strains were obtained from the International Life Sciences Institute North American Database (Cornell University, NY; 20). The strains used in this study were chosen after consultation with the database director, Dr. Martin Wiedmann. Cultures were maintained as frozen (-80°C) stock cultures, and working cultures of each strain were prepared by transferring 0.1 ml of a fresh overnight culture grown at 37°C in Brain Heart Infusion (BHI) broth (BBL, Becton Dickinson Co., Sparks, Md.) into 10 mL of fresh BHI and incubated at 37°C for 24 hours.

Preparation of Antimicrobial Treatments

Solutions of each organic acid (30% w/w) were prepared for use in meat formulations. A 30% sodium lactate solution (pH 6.3) was prepared by diluting a 60% (w/w) sodium L-lactate solution (Purac, Lincolnshire, IL) with an equal volume of distilled water. A 60% commercially available mixture of sodium lactate and sodium diacetate (pH 7.3) (1.875:0.125, respectively; Purasal Opti.Form SD 4 syrup, Purac, Lincolnshire, IL) was similarly diluted to 30% by addition of an equal volume of distilled water. A 30% sodium levulinate solution was prepared by adding 20% NaOH to a 98+% levulinic acid solution (Sigma Chemicals, St. Louis, MO) to obtain a pH of 6.59. The pKa of levulinic acid is 4.59, and attainment of a 2-pH unit difference due to the addition

of NaOH ensured that 99% of the levulinic acid was in the dissociated sodium salt form. The resulting levulinate was diluted with sufficient distilled water to produce a 30% solution with a pH of 6.6-6.7. The 30% solutions of antimicrobials were added to the meat formulations during preparation.

Turkey Roll Preparation

Whole fresh turkey breasts without tenders were purchased from Norbest (Midvale, UT). The common formulation consisted of the following as a percent of total weight in the formulation: turkey breast (60), water (34.11), sodium tripolyphosphate (0.5), salt (1.8), dextrose (1.99), sugar (0.8), and carrageenan (0.8). Twenty and one half kg of the fresh turkey breasts was ground through a 2.54 cm, two-blade knife, while the remaining 4 kg was ground through a 0.32 cm, four-blade knife. Both the coarsely ground and the finely ground meats were divided into six portions (~ 3.4 kg of the coarsely ground turkey meat and 0.68 kg of the finely ground turkey meat). One portion of coarsely ground meat and one portion of the finely ground meat were mixed manually in a food-grade vacuum bag for 10 minutes with the other ingredients and each of the antimicrobials or water, as appropriate. The formulations were then stuffed into 10 cm fibrous casings. Turkey breast prepared as described above with water instead of antimicrobial solutions served as a control throughout the experiment. Antimicrobials were added to the formulation in place of water and consisted of 2% sodium lactate, 2% combination of sodium lactate and sodium diacetate (Purasal Opti.Form SD 4), and either 1%, 2%, or 3% sodium levulinate.

The products were cooked for 2 hours at 54°C with 60% relative humidity (RH), followed by 2 hours at 65.5°C with 60% RH, and then 88°C with 60% RH until an internal temperature of 68°C was reached. Products were then showered with cold water for five minutes and stored overnight at 4°C. Meat samples were then cut into approximately 0.32 cm thick slices (about 25 grams per slice) and taken to the microbiology laboratory for inoculation with *L. monocytogenes*.

Bologna Preparation

The common bologna mixture consisted of the following as a percent of the total weight in the formulation: whole carcass beef (44.44), pork trim 50% fat (29.63), salt (2.04), Prague Powder (0.19), sodium erythorbate (0.04), phosphate (0.28), water as ice (12.32), sugar (0.38), white pepper (0.36), mustard (0.28), nutmeg (0.1), and garlic powder (0.04). The beef and pork were ground through a 2.54 cm plate. The beef, half of the ice, all the salt, Prague powder, and erythorbate were chopped to a fine paste (<5°C) before adding the pork trim meat, sugar, remaining ice and spices. Once all the ingredients were added, chopping continued until the batter reached 15°C. The batter was divided into six equal parts (~ 4.1 kg) and placed into plastic bags. The appropriate treatments, antimicrobials or water, were added so that the total percent in the formulation of water plus antimicrobial was 22.22%. Each was manually mixed and stuffed into 10 cm fibrous casings and then cooked at 54°C for 30 minutes, 65.5°C for 30 minutes, then 82°C at 100% relative humidity until the core temperature of the bologna reached 71°C. After cooking, the bologna were showered with cold water for 5 minutes and stored at 4°C overnight. The bologna was sliced into approximately 0.32 cm thick

slices (about 25 grams per slice) and taken to the microbiology laboratory for inoculation with *L. monocytogenes*.

Product Inoculation

Prior to inoculation of meat products, the 5 strains of *L. monocytogenes* were mixed according the procedure of Samelis et al. (43). Briefly, one-milliliter aliquots of each freshly grown strain (BHI, 24 hour, 37°C) were combined in a sterile, conical, 15-ml centrifuge tube. The mixture was centrifuged at 3,000 rpm for 15 minutes and washed twice with sterile phosphate-buffered saline (PBS; 8.45 mM of anhydrous Na_2HPO_4, 1.59 mM $NaH_2PO_4 \cdot H_2O$, and 145.45 mM NaCl, pH 7.4). The mixed culture was then serially diluted with PBS to obtain a concentration estimated to yield 10^2 to 10^3 CFU/cm^2 of product (Samelis et al., 2001). This composite inoculum was used to inoculate the meat products.

In all experiments, two slices of the RTE meat product from each treatment were placed into a 15.24 by 30.48 cm vacuum bag (Seward 6041 bags, London, England) and inoculated with 1 ml of composite inoculum under a biological safety hood. In all samples, the inoculum was distributed between the two slices of meat. Twenty-one bags (each containing two slices of product) were inoculated for each treatment so that triplicate samples could be analyzed for each treatment on each sampling day. All samples were vacuum-packaged (Minipack FASTVAC, Machine Runner, New York City, NY) and stored at 2°C for up to 12 weeks.

Cell Recovery and Enumeration

Recoverable colony forming units (CFU) were determined from triplicate bags of each treatment at 0, 2, 4, 6, 8, 10, and 12 weeks. Each sample bag was mixed with 100 ml of sterile PBS and placed in a stomacher (Seward 400, Seward Medical Limited, London SEI IPP, UK) and homogenized for 30 seconds on medium speed (230 rpm). Homogenates were serially diluted with sterile PBS and plated (0.1 ml), in duplicate, on RAPID'L.Mono selective agar (Bio-Rad Laboratories, Richmond, CA). The plates were incubated aerobically at 37°C for 48 hours. Colonies were counted from plates with counts between 30 and 300 colonies to calculate the total CFU on product for each treatment and time interval. Two independent trials of the entire turkey roll and bologna experiments were performed in the study.

Sensory Analysis

Open consumer taste panels were held to gather information on overall liking of turkey roll and bologna formulations using a hedonic scale from 1 to 9 (1=strongly disliked, 5=neither like nor dislike and 9=strongly like). The computer software, SIMS2000 (Sensory Computer Systems, Morristown,NJ), was used to generate random numbers for the labeling of the products, to compile a rotation plan, to write the questions, and to administer the questionnaire to the taste panel (see Appendix A). In addition to age and gender, panelists were asked about their general liking of the product being evaluated and their frequency of consumption. They rated their general liking of the product on a scale from 1 to 5 (1=dislike very much and 5=like very much), and their frequency of consumption from 1 to 5 (1= less than once a month and 5=more than once

a week). Panelists were also given the opportunity to provide written comments on each of the samples.

The turkey roll and bologna was prepared as described in the preceding sections about 5-7 days before the panels were held. The products were held at 4°C until the morning of the taste panel, at which time they were sliced (~0.32 cm thick) and cut into wedges. The wedges were rolled up croissant-style, and skewered with a toothpick to hold them in place.

Only five of the six samples were presented to the taste panels to avoid taste fatigue in the judges. In both the turkey roll and bologna taste panels, the judges were given the sample containing no antimicrobial, 2% sodium lactate, and 2% sodium lactate in combination with sodium diacetate. The samples containing sodium levulinate were selected for sensory analysis based on how effective they were at inhibiting *L. monocytogenes* in the microbiological experiments. In the turkey roll panel, judges were given 2% sodium levulinate and 3% sodium levulinate. They were not given a sample containing 1% sodium levulinate because it had not inhibited growth of *L. monocytogenes* during the 12-week study. For the bologna analysis, the consumers were given 1% sodium levulinate and 2% sodium levulinate. Product containing 3% sodium levulinate was not analyzed because it was no more inhibitory to *L. monocytogenes* than 1% or 2% sodium levulinate.

Statistical Analysis

Microbiological counts were analyzed for statistical significance using the proc mixed function, with a log transformation of the data, in Statistical Analysis Software

(SAS) version 9.1 (SAS Institute, Inc, Cary, NC). Analysis of variance was used to identify statistically significant differences at the 95% confidence level. Comparison of the means was made based on p-values (α=0.05) using the Tukey-Kramer adjustment to obtain differences of least means squares. The model included treatment type (water, 2% sodium lactate, 2% lactate-diacetate, and 1, 2, or 3% sodium levulinate) as the whole-plot factor and storage time (0, 2, 4, 6, 8, 10, and 12 weeks) as the sub-plot factor. Variance effects from repeated experiments were removed by blocking on trial. Turkey roll and bologna were analyzed separately.

The analysis of the sensory evaluation was performed by the SIMS2000 software, in conjunction with SAS, using the proc glm function with treatment as the main factor. ANOVA comparison of the means was made based on p-values (α=0.05) using the Tukey-Kramer adjustment to obtain differences of least means squares. The analysis was performed at the 95% confidence level.

RESULTS

Microbiological Data

Turkey Roll

Control samples, with only water added, supported prolific *L. monocytogenes* growth, as populations of the pathogen grew to 8 log CFU/cm^2 by 8 weeks of storage (Figure 1). The incorporation of the organic acid salts resulted in varying levels of *L. monocytogenes* inhibition relative to the control. The least effective treatment in the turkey breast, for both replicates, was the 2% sodium lactate, a current industry standard, followed by 1% sodium levulinate (Figure 1).

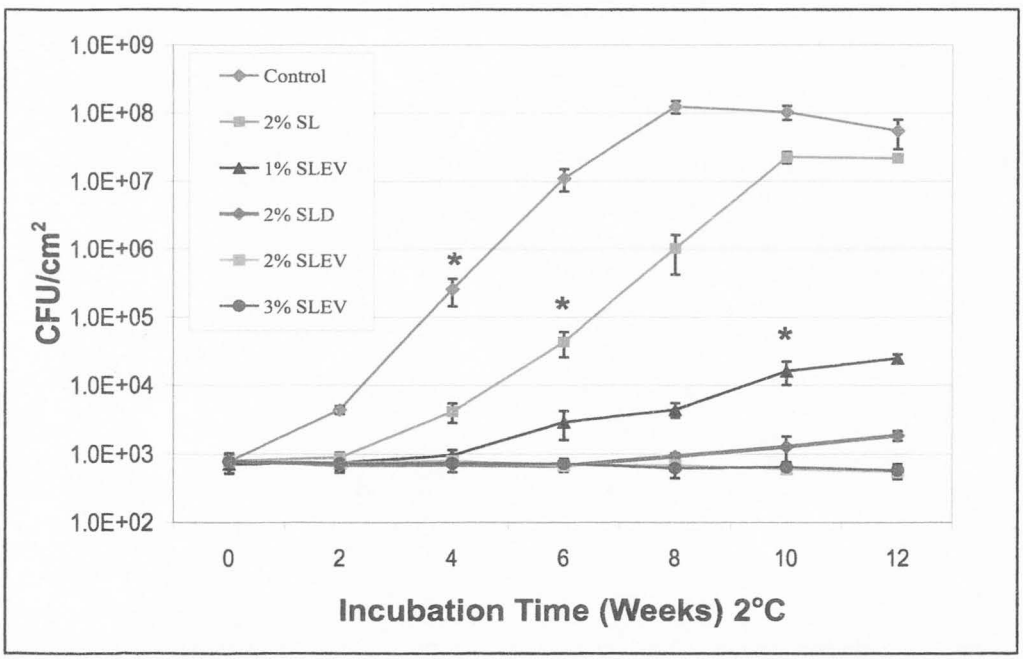

Figure 1. Growth of *L. monocytogenes* on turkey roll (log CFU/cm^2) incubated for up to 12 weeks at 2°C. * Indicates the first time when growth was significantly higher than original inoculation levels. Data represents the combined data from both trials. Figures illustrating data from individual replicates (or trials) can be found in Appendix B.

Significant growth (i.e., statistically greater than inoculation levels) occurred on samples containing sodium lactate after 6 weeks of storage ($P < 0.0001$), and after 10 weeks on samples containing 1% sodium levulinate ($P = 0.0047$). The remaining treatments (2% combination of sodium lactate and diacetate and 2% and 3% sodium levulinate) did not show significant growth above inoculation levels for the full 12 weeks of the study (Table 1). Analysis of variance table for the microbial analysis of the turkey roll can be found in Appendix C.

Table 1. Statistical analysis of microbiological data from turkey trials. Storage time at which treatments first showed significant growth from inoculation levels ($p<0.01$). *Indicates that treatments did not experience significant growth from inoculation levels for the full twelve weeks. Treatments sharing the same superscript letters are not significantly different at $p>0.01$. For a complete list of comparison of treatment types see Appendix D.

Treatment Type	Storage Time (weeks)	Mean Growth
No Antimicrobial	4	4.23E+07^{a}
2% Sodium Lactate	6	6.50E+06^{b}
Sodium Lactate + Diacetate*	12 +	9.84E+02^{cd}
1% Sodium Levulinate	10	7.27E+03^{c}
2% Sodium Levulinate*	12 +	6.88E+02^{d}
3% Sodium Levulinate*	12 +	6.82E+02^{d}

Bologna

The control bologna (that containing no antimicrobial) allowed significant growth by 8 weeks of incubation at 2°C reaching 6 log CFU/cm^2 (Figure 2). Growth of *L. monocytogenes* was not detected on any other treatment over the 12 weeks storage at 2°C. It follows that overall bacterial growth was greatest on control samples, with no

significant differences among the other treatments (Table 2). A complete table (Table D2) of treatment type comparisons and adjusted p-values can be found in Appendix D.

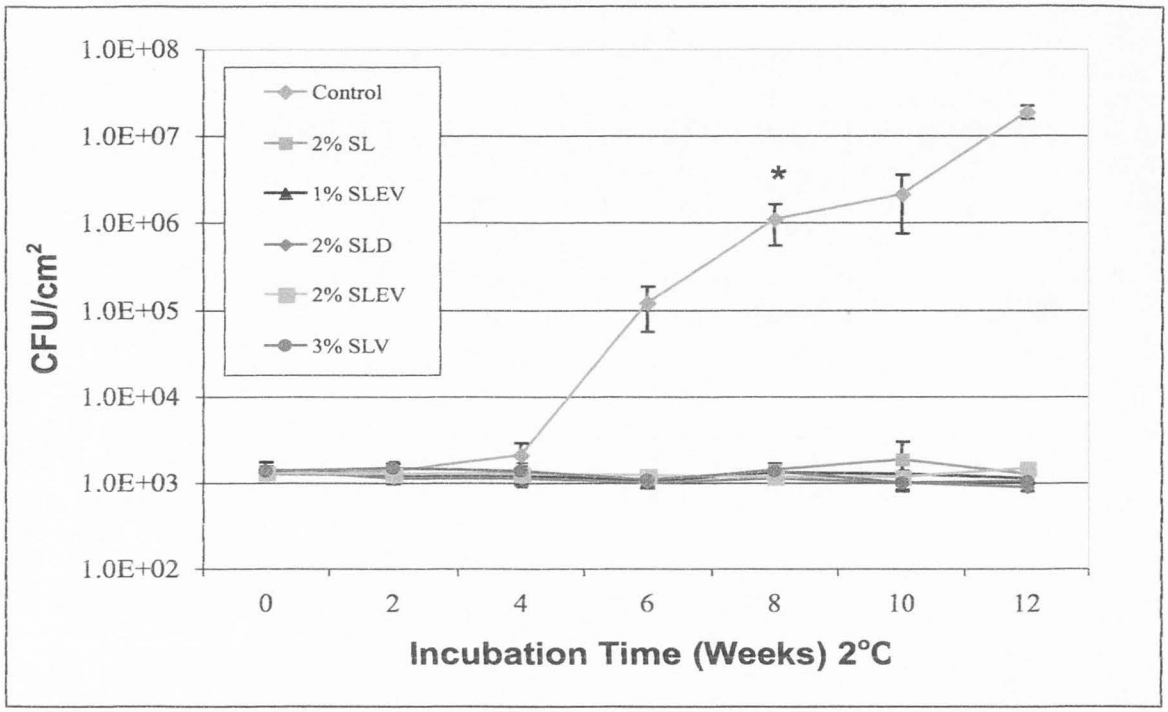

Figure 2. Growth of *L. monocytogenes* on bologna incubated for up to 12 weeks at 2°C. * Indicates the time when growth was significantly higher than inoculation levels. Data represents the combined data from both trials. Figures illustrating data from individual replicates (or trials) can be found in Appendix B.

Analysis of variance table for the microbial analysis of the bologna can be found in appendix C.

Table 2. Statistical analysis of microbiological data from bologna trials. Storage time for which treatments first showed significant growth from initial inoculation levels ($p<0.01$). *Indicates that treatments did not experience significant growth from inoculation levels for the full twelve weeks. Treatments sharing the same superscript letter are not significantly different at $p>0.01$. For a complete list of comparison of treatment types see Appendix D.

Treatment Type	Storage Time (weeks)	Mean Growth
No Antimicrobial	8	3.12E+06[a]
2% Sodium Lactate*	12 +	1.33E+03[b]
Sodium Lactate + Diacetate*	12 +	1.10E+03[b]
1% Sodium Levulinate	12 +	1.22E+03[b]
2% Sodium Levulinate*	12 +	1.25E+03[b]
3% Sodium Levulinate*	12 +	1.23E+03[b]

Sensory Analysis

Five samples were evaluated by an open consumer taste panel. For turkey roll evaluation, the five samples presented to the panelists to taste and score according to overall liking of the product included the control with no antimicrobials, 2% sodium lactate, the combination of sodium lactate and sodium diacetate, 2% sodium levulinate and 3% sodium levulinate. For bologna, the five samples included the control, 2% sodium lactate, the combination of sodium lactate and sodium diacetate, and 1% and 2% sodium levulinate.

There were 132 consumers who participated in the sensory panel for the turkey roll and 112 for the bologna. Of those who participated in the turkey roll panel, 66 were female and 66 were male. There was a wide range in age among the participants, with the majority being in the 18 to 25 age group (Figure 3). Similar demographic data was found among the participants of the bologna sensory panel. There were 58 females and 54 males, most of whom were in the 18 to 25 age group (Figure 3).

The members of the turkey roll panel moderately liked turkey roll (mean=4.33) and consumed it about once a week (mean=4.33). The members of the bologna sensory panel noted that they neither liked nor disliked bologna (mean=3.58) and consumed it less than once a month (mean=1.56).

There was no significant difference in the overall liking of the turkey roll (P = 0.19) or the bologna (P = 0.42) (Table 3).

Table 3. Statistical analysis of mean sensory scores for turkey and bologna samples. Treatments with the same superscript letter are not significantly different at p > 0.05.

	Control	2% Sodium Lactate	Sodium Lactate plus Diacetate	1% Sodium Levulinate	2% Sodium Levulinate	3% Sodium Levulinate	p-Value
Turkey	6.8[a]	6.9[a]	6.8[a]	0	6.6[a]	6.6[a]	0.19
Bologna	6.2[b]	6.2[b]	6.2[b]	6.0[b]	6.0[b]	0	0.42

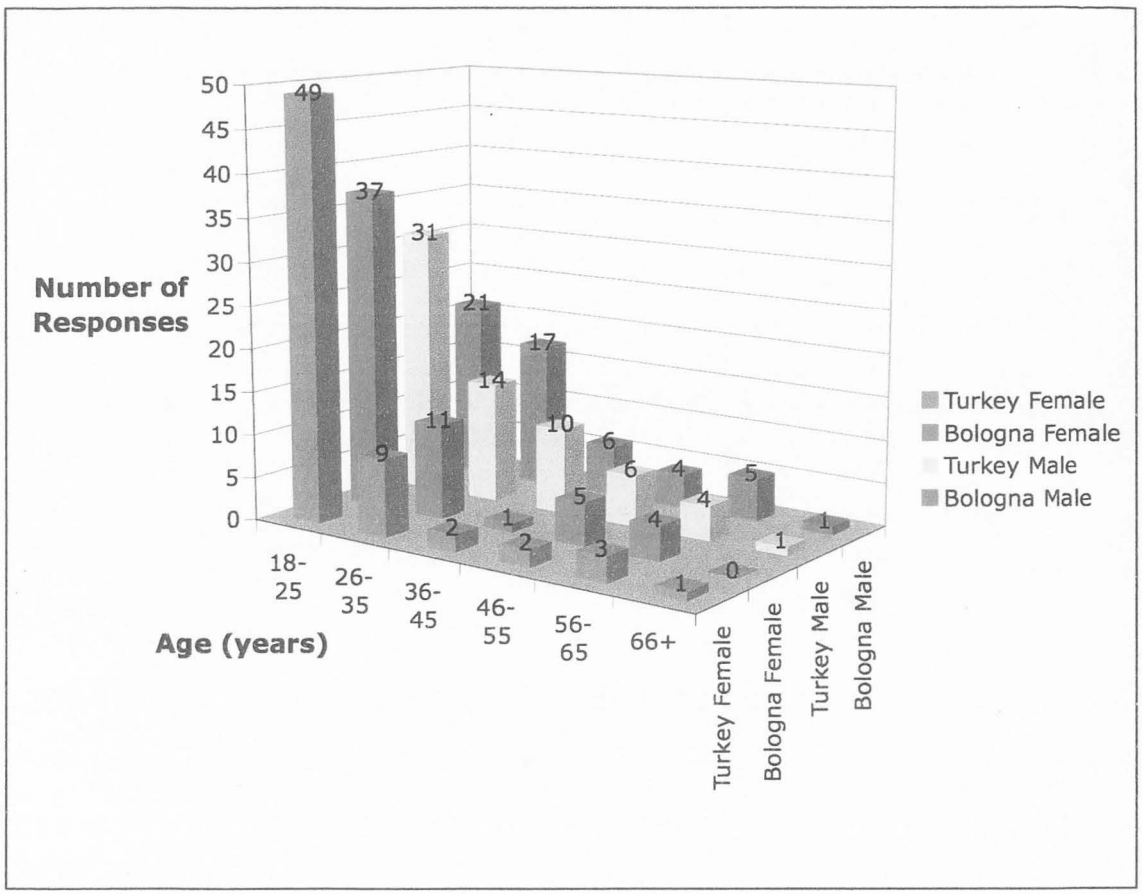

Figure 3. Distribution of age and gender of the participants of the sensory panels for turkey roll and the bologna.

Space was provided on the questionnaire for the judges to provide written comments on each of the samples. Of the 132 panelists who tasted turkey roll, 56 (42%) provided at least one comment on at least one sample. Similarly, in the bologna analysis 56 panelists (50%) provided comments on at least one sample. For both the turkey roll and bologna individually, there were more negative comments than positive (65% negative). The majority of the provided comments fell into one of five categories for the turkey roll: relating to texture, relating to flavor, relating to aftertaste, relating to saltiness, or relating to juiciness. The four categories of key words that were noted for

the bologna were the same as the turkey roll with the exception of juiciness. Due to a large number of the occurrences of the descriptor "salty", this was tallied as a separate category (Figures 4 and 5).

Comments were evaluated for certain key words or phrases in each category, and total occurrences were tallied (Figures 4 and 5). The most common texture descriptors for both meat samples were "soft," "slimy," "rubbery," "chewy," "good," and "tough." Flavor descriptors included "funny," "metallic," "old," "nice," "meaty," "honey," "smoked," and "spicy." Aftertaste descriptors used were simply "good" or "bad", and in the turkey panel, juiciness was described as either "juicy" or "dry."

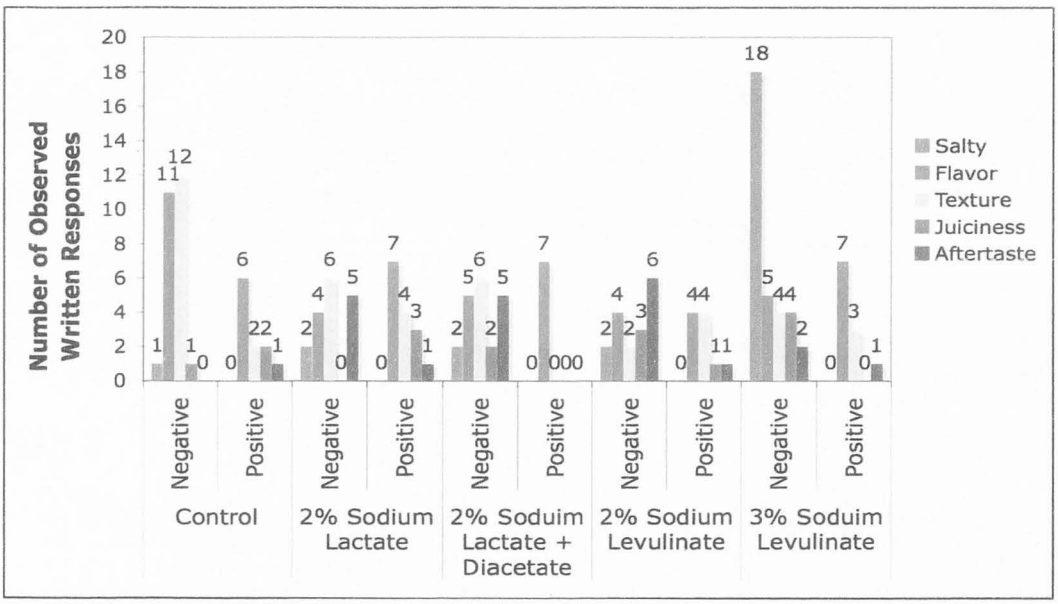

Figure 4. Negative and positive comments in turkey roll sensory analysis. Bar values are total observations per sample, where 56 of the 132 panelists provided at least one written comment.

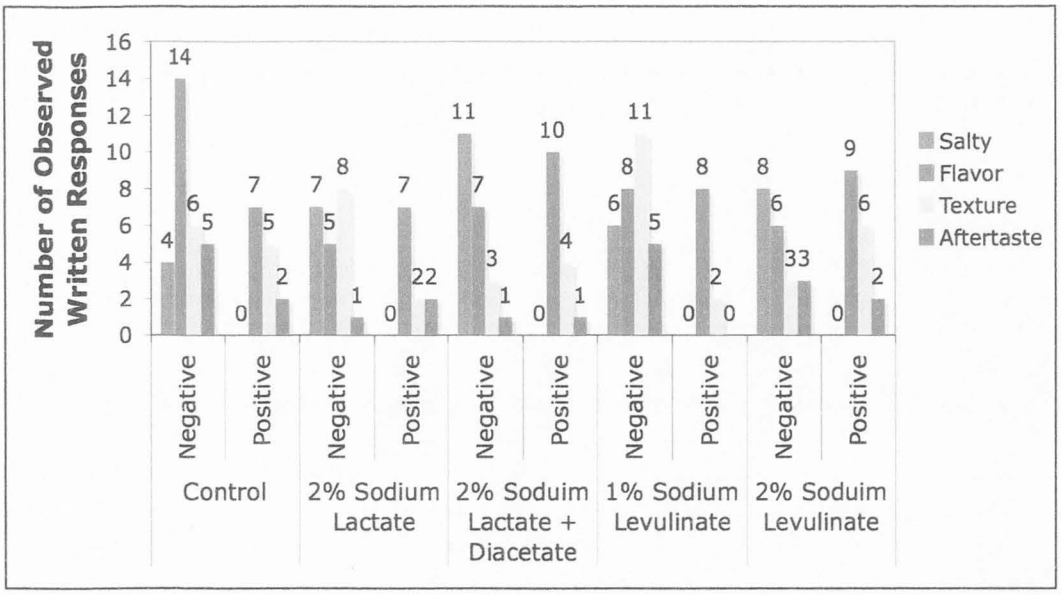

Figure 5. Negative and Positive comments in bologna sensory analysis. Bar values are total observations per sample, where 56 of the 112 panelists provided at least one written comment.

DISCUSSION

Turkey Roll

The effects of six additives (water, 2% sodium lactate, 2% sodium lactate combined with sodium diacetate, 1%, 2%, and 3% sodium levulinate) on the growth of *L. monocytogenes* were studied. As expected, the samples containing water rather than an antimicrobial allowed the most growth of the pathogen; however, cell death was not noted in any treatment. Growth of *L. monocytogenes* in turkey roll was more inhibited by the addition of sodium levulinate at any of the three levels tested than by the addition of 2% sodium lactate, a current industry standard. In fact, sodium lactate used alone only inhibited proliferation of *L. monocytogenes* for less than six weeks of storage, and in this study did not meet the FSIS requirements to qualify as an additive to control the growth of *L. monocytogenes* (18).

The 1% sodium levulinate was the next least inhibitory, yet it was not significantly different from the combination of sodium lactate and sodium diacetate, another industry standard. Furthermore, sodium levulinate at 2 or 3% was at least as inhibitory to *L. monocytogenes* growth as the combination of sodium lactate and sodium diacetate. Specifically, both 2 and 3% sodium levulinate inhibited the pathogen for up to 12 weeks at 2°C. In a study designed to compare various antimicrobials in their effectiveness at inhibiting *L. monocytogenes*, Barmpalia et al. (3) reported that 1.75% sodium lactate combined with 0.25% sodium diacetate was the most effective treatment. Similarly, Samelis et al. (44) illustrated that 1.75% sodium lactate in combination with 0.25% glucono-delta-lactone provided comparable inhibition of *L. monocytogenes* as the

combination of sodium diacetate with sodium lactate. These results show that the combinations of various antimicrobials are often more effective than either one used individually. This study found that the combination of lactate and diacetate worked better than lactate alone, but not better than sodium levulinate.

It is important to note that growth of *L. monocytogenes* occurred on the samples containing the combination of lactate and diacetate between 6 and 8 weeks and continued to grow through the end of the trial. This trend of growth indicates that the combination of lactate and diacetate may not meet the FSIS requirements in a longer shelf-life study and reinforces the need for better antimicrobials in non-cured RTE meats.

Bologna

As was the case in the turkey roll experiments, *L. monocytogenes* proliferated on the control bologna, but there was no growth of *L. monocytogenes* on any treated bologna during the twelve weeks. Similarly, Barmpalia et al. (3) found that growth of this pathogen reached nearly 10^9 CFU/cm^2 on bologna containing no antimicrobials after 90 days at both 10°C and 4°C, but this growth was inhibited by 1.75% sodium lactate combined with 0.25% diacetate.

It is notable that all the antimicrobials prevented growth *of L. monocytogenes* on the bologna, but not on the turkey roll. This can be attributed to the fact the bologna is a cured meat product, and therefore contains nitrite and a higher salt concentration. Nitrite is used in cured meats to preserve desirable meaty flavor, prevent warmed over flavor, and give them their characteristic bright reddish pink color. However, one of the most

important incentives for including nitrite and salt is to inhibit microbial growth, particularly that of *Clostridium botulinum* (1).

The inhibitory effects of the antimicrobial treatments, in addition to the nitrite and high salt concentration in the bologna formulation, fully prevented growth of *L. monocytogenes* at 2°C. This observation is consistent with the concept that multiple barriers in food protection are more effective than using individual obstacles by causing simultaneous and variable damage and stress to the bacterial cells (29, 52). It becomes too energetically demanding for injured cells to maintain homeostasis and repair various damages caused by multiple hurdles, which causes extended lag phase or cell death. Although we did not detect cell death of the *L. monocytogenes* in any treatment, a considerably longer lag phase was apparent in the bologna versus turkey controls. Clearly, the strong synergistic effects of the combination of the antimicrobials and the nitrite in the bologna formulation were more effective than they would be without the presence of the nitrite.

Sensory Analysis

A sensory panel consisting of anonymous consumers rated turkey roll and bologna samples for their overall liking of the products. The panelists had no preferences among the various samples in both the turkey roll and the bologna analyses ($p > 0.05$). The addition of lactate has previously been reported to have positive effects on the flavor, shelf life, color, tenderness, and juiciness of various meat products (15, 58), but this was not observed here. Some consumers commented on liking the flavor of the turkey roll and bologna containing the salt forms of the organic acid antimicrobials, while indicating

that the control was too bland, but this did not significantly impact overall liking. The control samples in both the turkey roll and bologna panels received the most negative comments on the flavor.

Many panelist consumers commented that the 3% sodium levulinate was noticeably more salty than the other samples, but again, this did not affect liking. The turkey panelists were not given a sample containing 1% sodium levulinate because it was significantly less effective in control of the pathogen in the microbiological analysis, and not likely to be approved by USDA as a control for *L. monocytogenes* for use in RTE foods.

In the bologna panel, one of the most frequent comments was that the control, containing no antimicrobial, was very bland and that all of the other samples had a better flavor. As was found in the turkey roll panel, the higher level of sodium levulinate (2%) was often described as very salty, but this did not have an affect on their liking of the product. Again, there would be no microbiological benefit by using the higher level of sodium levulinate when the 1% in the bologna formulation gave an equivalent level of protection against the pathogen for up to 12 weeks.

The sensory analyses indicate that sodium levulinate is comparable in liking to current industry standards, and that it does not have any negative impact on the organoleptic characteristics of the products when used at appropriate levels.

Possible Mechanisms for Levulinate Inhibition

There are many mechanisms thought to contribute to the effectiveness of weak organic acids as antimicrobials in RTE meats. Three putative mechanisms by which acid

anions inhibit bacterial growth are acidification of the cytoplasm (14, 19, 26, 28, 36, 53, 55); accumulation of anions in the cytoplasm (40, 41, 42); and disruption of the lipid membrane (19, 25, 54).

It is clear that undissociated (protonated) acid molecules diffuse into the cytoplasm through the cell membrane (28, 36, 53). In the cytoplasm, the weak acid dissociates into anions and protons. The proton is pumped back across the membrane at an energy cost to the cell, while the anion remains in the cytolplasm. If the rate of proton accumulation exceeds that of efflux systems, the internal pH begins to fall and the cytoplasm becomes more acidic. This acidification of the cytoplasm may repress growth by inhibiting glycolysis (14, 26), preventing active transport (19), or by interfering with signal transduction (55).

Increasing evidence suggests that accumulation of anions within the cell may be more inhibitory to growth than release of the proton, especially in a mildly acidic environment as is found in most RTE meat products (40, 41, 42). Anion accumulation may inhibit various metabolic functions of the cell. Specifically, accumulation of acetate anion caused intracellular methionine depletion and the accumulation of homocysteine, a toxic methionine intermediate in *E. coli* (40). High concentration of anion significantly increases the osmolarity of the cytoplasm, causing potentially lethal increases in turgor pressure as the flow of water into the cell increases. However, the cell compensates for this by reducing the concentrations of other solutes in the cytoplasm. In particular, glutamate, which is the largest single pool of osmotically active anions (constituting 25% of the total anion pool in some cells) (39).

Undissociated weak acids are known to partition into membrane lipid bilayers at concentrations that are dependant upon their lipophilicity (i.e., their ability to dissolve in fats, oils, lipids, and non-polar solvents). There is a good correlation between the inhibition by the weak acids and their respective lipophilicities (54). In theory, small organic molecules interfere with membrane proteins by inhibiting the transport of substrate molecules into cells, leading to growth inhibition (25).

In summary, the preservation of foods by the addition of weak acids may be due to the concerted effects of various possible mechanisms. The available evidence indicates that weak acids inhibit microorganisms by lowering the internal pH of the cells, interrupting membrane functions, and interfering with the osmotic properties and metabolic functions resulting from the accumulation of anions in the cytoplasm.

It is well known that different organic acids vary considerably in their inhibitory effects. This study showed that sodium levulinate is more effective at inhibiting the growth of *L. monocytogenes* than either sodium lactate used alone or in combination with sodium diacetate. *L. monocytogenes* produces and utilizes lactic acid in various pathways, including in the production of pyruvate and NADH. Pyruvate is converted into acetyl-coenzyme A (acetyl-CoA), which is the main input for a series of energy-producing reactions in the Krebs cycle. Pyruvate can be converted to carbohydrates via gluconeogenesis, to fatty acids or energy through acetyl-CoA, to the production of the amino acid alanine, and to ethanol. Therefore, it unites several key metabolic processes.

Acetic acid is produced in pathways such as mixed acid fermentation, ornithine biosynthesis, and homocysteine biosynthesis; it is utilized in ethanol fermentation and the production of acetyl-CoA. Acetyl-CoA is an important molecule in metabolism, and is

used in many biochemical reactions. Its main use is to convey the carbon atoms within the acetyl group to the Krebs Cycle to be oxidized for energy production. Because lactate and acetate are both compounds found in vital metabolic pathways of *L. monocytogenes*, it is our hypothesis that the pathogen is better equipped to handle these anions when they begin accumulating in the cytoplasm than it is levulinate, so accumulation of levulinate anion is more debilitating to the pathogen.

Future Work

There are many studies that could further determine the potential of levulinic acid as an antimicrobial food additive. These include looking at sodium levulinate in combination with sodium lactate or sodium diacetate, investigating the mechanism by which levulinic acid inhibits *L. monocytogenes*, its potential in other areas of food processing, such as carcass washes, and its effectiveness on other types of foods (i.e. fresh fruits and vegetables).

As shown in this study and in many previous studies, antimicrobial ingredients become more effective when they are used in combination with other organic acids. When sodium lactate is in combination with sodium diacetate it becomes significantly more inhibitory to *L. monocytogenes* than when used alone. Combinations of sodium levulinate with lactate or acetate could possibly increase the antilisterial effectiveness of levulinate alone. It would be extremely beneficial to study these different combinations because sodium levulinate alone (2 and 3%) did not allow any growth of *L. monocytogenes*; therefore, if any of them strengthened the effectiveness of sodium

levulinate, it might result in actual cell death, not just inhibition of the pathogen, or require an even lower level of levulinate.

As discussed previously, there are many possible mechanisms believed to contribute to how these weak organic acids inhibit microbial growth. The current study shows that levulinic acid is much more effective than either industry standard alone. Knowledge of the mechanisms by which levulinic acid inhibits *L. monocytogenes* would enable us to understand why it is a more effective antimicrobial than the other industry standards. This could lead to finding other acids that could be as effective as levulinic acid at inhibiting bacteria in food. It could also tell us something about what other bacteria would be susceptible to inhibition by levulinic acid. It would also tell us a lot about whether it is capable of causing cell death or if it acts only to inhibit the growth of microorganisms.

There are many steps in food processing in which antimicrobials are utilized, besides as an ingredient. One such critical step in meat processing is carcass washing to eliminate and inhibit pathogenic and spoilage bacteria on the surface of the meat. Currently the industry uses acid washes such as sodium lactate and sodium diacetate – similar to what was used in this study; thus, sodium levuliniate could prove to be an effective carcass wash. Another similar application of sodium levulinate as an antimicrobial in the food industry would be its use as a wash for fresh fruits and vegetables.

CONCLUSIONS

Sodium levulinate was significantly more effective at inhibiting growth of *L. monocytogenes* in turkey roll than current industry standard of sodium lactate. Again, the 2% sodium lactate did not meet the FSIS requirements during this study. Used at levels of 2% or 3%, sodium levulinate was at least as inhibitory to the growth of the pathogen on both turkey roll as the combination of sodium lactate and sodium diacetate, another current industry standard. In the bologna, all three levels of sodium levulinate were just as inhibitory as 2% sodium lactate alone or in combination with sodium diacetate.

Sodium levulinate did not have a negative impact on the overall liking of either RTE product. The turkey roll and bologna containing sodium levulinate, at any concentration, were liked as well as the other treatments tested. One benefit of utilizing sodium levulinate in the control of *L. monocytogenes* in RTE meat products is the option of rotating it with other antimicrobials to avoid strain resistance. Another is that sodium levulinate could prove to be an even stronger inhibitor of *L. monocytogenes* when used in combination with another antimicrobial. This study demonstrates that 2% or greater sodium levulinate suppresses the growth of *Listeria monocytogenes* to less than 1 log during refrigerated storage of both cured and uncured vacuum packaged RTE meats. Therefore, sodium levulinate should qualify as an antimicrobial under FSIS verification standards. Additionally, sodium levulinate does not have deleterious effects on the flavor of the products.

REFERENCES

1. **Aberle, E.D., J.C. Forrest, D.E Gerrard, and E.W. Mills.** 2001. Principles of meat processing, p. 117-153. *In* Principles of meat science. Fourth edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

2. **Ahamad, N. and E.H. Marth.** 1989. Behavior of *Listeria monocytogenes* at 7, 13, 21, and 35°C in tryptose broth acidified with acetic, citric or lactic acid. J. Food Prot. **52**:688-695.

3. **Barmpalia, I. M., K. P. Koutsoumanis, I. Geornaras, K. E. Belk, J. A. Scanga, P. A. Kendall, G. C. Smith, and J. N. Sofos.** 2005. Effect of antimicrobials as ingredients of pork bologna for *Listeria monocytogenes* control during storage at 4 or 10° C. Food Microbiol. **22**:205-211.

4. **Bedie, G. K., J. Samelis, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith.** 2001. Antimicrobials in the formulation to control *Listeria monocytogenes* post-processing contamination on frankfurters stored at 4°C in vacuum packages. J. Food Prot. **64**:1949-1955.

5. **Blom, H., E. Nerbrink, R. Dainty, T. Hagtvedt, E. Borch, H. Nissen, and T. Nesbakken.** 1997. Addition of 2.5% lactate and 0.25% acetate controls growth of *Listeria monocytogenes* in vacuum packaged, sensory acceptable servelat sausage and cooked ham stored at 4°C. Int. J. Food Microbiol. **38**:71-76.

6. **Bone, R.C.** 1991. The pathogenesis of sepsis. Ann. Intern. Med. **115**:457-469.

7. **Buncic, S., C. M. Fitzgerald, R. G. Bell, and J.A. Hudson.** 1995. Individual and combined listericidal effects of sodium lactate, potassium sorbate, nisin, and curing salts at refrigeration temperature. J. Food Safety **15**:247-264.

8. **Cabanes, D., P. Dehoux, O. Dussurget, L. Frangeul, and P. Cossart.** 2002. Surface proteins and the pathogenic potential of *Listeria monocytogenes*. TRENDS Microbiol. **10**:238-245.

9. **Center for Disease Control and Prevention**. 1998. Multistate outbreak of listeriosis-United States. M.M.W.R. **47**:1085-1086.

10. **Center for Disease Control and Prevention**. 1999. Multistate outbreak of listeriosis-United States. M.M.W.R. **47**:1117-1118.

11. **Center for Disease Control and Prevention**. 2000. Multistate outbreak of listeriosis-United States. M.M.W.R. **49**:1129-1130.

12. **Center for Disease Control and Prevention**. 2002. Public Health Dispatch: Outbreak of listeriosis-Northeastern United States. M.M.W.R. **51**:950-951.

13. **Center for Disease Control and Prevention**. 2005. Meningococcal Disease. [Online] Accessed 15 Mar. 2007. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/meningococcal_g.htm

14. **Davidson, P. M.** 2001. Chemical preservatives and natural antimicrobial compounds, p. 593-627. *In* M.P. Doyle, L.R. Buchchat, and T.J. Montville. (ed.), Food Microbiology- Fundamentals and Frontiers. 2nd ed. American Society for Microbiology, Washington, DC.

15. **Eckert, L. A., J. V. Maca, R. K. Miller and G. R. Acuff.** 1997. Sensory, microbial and chemical characteristics of fresh aerobically stored ground beef containing sodium lactate and sodium propionate. J. Food Science **62**:429-433.

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

17. **Farber, J. M., and P. I. Peterkin.** 1999. Incidence and behavior of *Listeria monocytogenes* in meat products, p. 505-564. *In* E.T. Ryser and E.H. Marth (ed.), Listeria, listeriosis and food safety. Marcel Dekker Inc., New York.

18. **Food Safety and Inspection Service.** 2002. USDA directive to reduce *Listeria monocytogenes* in ready-to-eat meat and poultry products. www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/10240.3.pdf

19. **Freese, E., C. W. Sheu, and E. Galliers.** 1973. Function of lipophilic acids as antimicrobial food additives. Nature **241**:321-325.

20. **Fugett, E., E. Fortes, C. Nnoka, and M. Wiedmann.** 2006. International Life Sciences Institute North America *Listeria monocytogenes* strain collection: development of standard *Listeria monocytogenes* strain sets for research and validation studies. J. Food Prot. **69:**2929-2938.

21. **Ghorpade, V. and M. Hanna.** 2007. Levulinic acid Industrial Applications. [Online] Accessed 25 Feb. 2007. http://www.plannedprofitablegrowth.com/levulinic.html

22. **Glaser, P., L. Frangeul, C. Buchrieser, C. Rusniok, A. Amend, F. Baquero, P. Berche, H. Bloecker, P. Brandt, T. Chakraborty, A. Charbit, F. Chetouani, E. Couve, A. de Daruvar, P. Dehoux, E. Domann, G. Dominguez-Bernal, E. Dechaud, E. Durant, O. Dussurget, K.D. Entian, H. Fsihi, F. Garcia-Del Portillo, P. Garrido, L. Gautier, W. Goebel, N. Gomez-Lopez, T. Hain, J. Hauf, D. Jackson, L. M. Jones, U. Kaerst, J. Kreft, M. Kuhn, F. Kunst, G. Kurapkat, E. Madueno, A. Maitournam, J. Mata Vicente, E. Ng, H. Nedjari, G. Nordsiek, S. Novella, B. de Pablos, J.C. Perez-Diaz, R. Purcel, B. Remmel, M. Rose, T. Schlueter, N. Simoes, A. Tierrez, J.A. Vasquez-Boland, H. Voss, J. Wehland, and P. Cossart.** 2001. Comparative genomics of *Listeria* species. Science. **294:**849-852.

23. **Glass, K., D. Preston, and J. Veesenmeyer.** 2007. Inhibition of *Listeria monocytogenes* in turkey and pork beef bologna by combinations of sorbate, benzoate, and propionate. J. Food Prot. **70**:214-217.

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

25. **Hirshfield, I. N., S. Terzulli, and C. O'Byrne.** 2003. Weak organic acids: a panoply of effects on bacteria. Sci. Prog. **86:**245-269.

26. **Krebs, H. A., D. Wiggins, M. Stubs, A. Sols, and F. Bedoya.** 1983. Studies on the mechanism of the antifungal action of benzoate. Biochem. J. **214**:657-663.

27. **Kulesa, G.** 1999. Manufacture of industrial chemicals from levulinic acid: a new feedstock for the chemicals industry. [Online] Accessed 8 Nov. 2006. http://www.oit.doe.gov/IOF/chemicals

28. **Lambert, R. J., and M. Stratford.** 1999. Weak acid preservatives: modeling microbial inhibition and response. J. Appl. Microbiol. **86**:157-164.

29. **Leistner, L.** 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. **55**:181-186.

30. **Maca, J. V., R. K. Miller, and G. R. Acuff.** 1997. Microbiological, sensory ad chemical characteristics of vacuum-packaged ground beef patties treated with salts of organic acids. J. Food Sci. **62**:591-596.

31. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food related illness and death in the United States. Emerg. Inf. Dis. **5**:607-625.

32. **Mbandi, E. and L. A. Shelef.** 2001. Enhanced inhibition of *Listeria monocytogenes* and *Salmonella enteritidis* in meat by combinations of sodium lactate and diacetate. J. Food Prot. **64**:640-644.

33. **Mbandi, E. and L. A. Shelef.** 2002. Enhanced antimicrobial effects of combinations of lactate and diacetate on *Listeria monocytogenes* and *Salmonella* spp. in beef bologna. Int. J. Food Microbiol. **76**:191-198.

34. **Murray, E. G. D., R. A. Webb, and M. B. R. Swan.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. J. Pathol. Bacteriol. **29**:407-439.

35. **Renzoni, A., A. Klarsfeld, S. Dramsi, and P. Cossart.** 1997. Evidence that PrfA, the pleiotropic activator of virulence genes in *Listeria monocytogenes*, can be present but inactive. Infect. Immun. **65**:1515-1518.

36. **Ricke, S. C.** 2003. Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. Poultry Sci. **82**:632-639.

37. **Robbins, J.R., A.I Barth, H. Marquis, E.L. de Hostos, W.J. Nelson, and J.A. Theriot.** 1999. *Listeria monocytogenes* exploits normal host cell processes to spread from cell to cell. J. Cell Bio. **146:**1333-1349.

38. **Roberts, D.** 1994. *Listeria monocytogenes* and food: The U.K. approach. Dairy, Food and Environ. Sanit. 14, 198, **200:**202-204.

39. **Roe, A. J., D. Mclaggan, I. Davidson, C. O'Byrne, and I.R. Booth.** 1998. Perturbation of anion balance during inhibition of growth of *Escherichia coli* by weak acids. J. Bacteriol. **180:**767-772.

40. **Roe, A. J., C. O'Byrne, D. McLaggan, and I. R. Booth.** 2002. Inhibition of *Escherichia coli* growth by acetic acid: a problem with methionine biosynthesis and homocyseine toxicity. Microbiol. **148**:2215-2222.

41. **Russell, J. B.** 1992. Another explanation for the toxicity of fermentation acids at low pH; anion accumulation verses uncoupling. J. Appl. Microbiol. **73**:363-370.

42. **Russell, J. B., and F. Diez-Gonzalez.** 1998. The effects of fermentation acids on bacterial growth. Adv. Microb. Physiol. **39**: 205-234.

43. **Samelis J., J. N. Sofos, M. L. Kain, J. A. Scanga, K. E. Belk, and G. C. Smith.** 2001. Organic acids and their salts as dipping solutions to control *Listeria monocytogenes* inoculated following processing of sliced pork bologna stored at 4°C in vacuum packages. J. Food Prot. **64**:1722-1729.

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

45. **Schlyter, J. H., A. J. Dengan, J. Loeffelholz, K. A. Glass, and J. B. Luchansky.** 1993a. Evaluation of sodium diacetate and ALTA™ 2341 on viability of *Listeria monocytogenes* in turkey slurries. J. Food Prot. **56**:808-810.

46. **Schlyter, J. H., A. J. Dengan, J. Loeffelholz, K. A. Glass, and J. B. Luchansky.** 1993b. The effects of diacetate with nitrite, lactate, or pedoicin on the viability of *Listeria monocytogenes* in turkey slurries. Int. J. Food Microbiol. **19**:271-281.

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

48. **Sheehan, B., A. Klarsfeld, T. Msadek, P. Cossart.** 1995. Differential activation of virulence gene expression by PrfA, the *Listeria monocytogenes* virulence regulator. J. Bacteriol. **177**:6469-6476.

49. **Shelef, L. A.** 1994. Antimicrobial effects of lactates, a review. J. Food Prot. **57**:445-450.

50. **Shelef, L. A., and L. Addala.** 1993. Inhibition of *Listeria monocytogenes* and other bacteria by sodium diacetate. J. Food Safety **14**:103-115.

51. **Shelef, L. A., and Q. Yang.** 1991. Growth suppression of *Listeria monocytogenes* by lactates in broth, chicken and beef. J. Food Prot. **54**:283-287.

52. **Sofos, J.N.** 1993. Current microbiological considerations in food preservation. Int. J. Food Microbiol. **19**:87-108.

53. **Stratford, M., and A. H. Rose.** 1986. Transport of sulfur dioxide by *Saccharomyces cerevisiae.* J. Gen. Microbiol. **132**:1-6.

54. **Stratford, M., and P.A. Anslow.** 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. Lett. Appl. Microbiol. **27**:203-206.

55. **Thevelein, J. M.** 1994. Signal transduction in yeast. Yeast **10**:1753-1790.

56. **Tompkin, R. B.** 2002. Control of *Listeria monocytogenes* in the food processing environment. J. Food Prot. **65**:709-725.

57. **Vasavada M., C. E. Carpenter, and D. P. Cornforth.** 2003. Sodium levulinate and sodium lactate effects of microbial growth and stability of fresh pork and turkey sausages. J. Muscle Foods **14**:119-129.

58. **Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith, and N. C. Speer.** 2000. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. J. Animal Sci. **78**:952-957.

59. **Weaver, R. A. and L. A. Shelef.** 1992. Antilisterial activity of sodium, potassium or calcium lactate in pork liver sausage. J. Food Safety **13**:133-146.

60. **Wederquist, H. J., J. N. Sofos, and G. R. Schmidt.** 1994. *Listeria monocytogenes* inhibition in refrigerated vacuum packaged turkey bologna by chemical additives. J. Food Sci. **59**:498-500, 516.

APPENDICES

Appendix A

Sensory Analysis Ballots and Rotation Plans

Welcome to the Sensory Evaluation Laboratory!

Please click on the hand above to proceed.

Before you start tasting the samples, please take a few minutes to answer the following questions about yourself. Please check the appropriate boxes.

1.) What is your GENDER?

Male	Female

2.) What is your AGE?

18-25	26-35	36-45	46-55	56-65	over 65

3.) In general, how do you rate your liking of the meat product being tested today (either turkey breast roll or bologna)?

Dislike Very Much	Moderately Dislike	Neither Like nor Dislike	Moderately Like	Like Very Much

3.) In general, how often do you consume the product being tested today?

Less Than Once a Month	About Once a Month	2-3 Times a Month	Once a Week	More Than Once a Week

Thank you!!! We are now ready to begin the sensory test.

Figure A1. Sample taste panel ballot: demographics section. Used for both turkey roll panel and bologna panel.

Read Instructions Carefully Before Starting

Today you will be sampling five meat samples.

Please taste each sample in the order you are prompted. Please match the number on the computer screen prompt to the sample number on the ballot.
As you sample each piece of meat, please score it according to your overall liking of the sample. Please score each sample according to the following scale and mark your choice in the corresponding box.

REMEMBER to rinse your mouth between samples!

9 = Like extremely
8 = Like very much
7 = Like moderately
6 = Like slightly
5 = Neither like nor dislike
4 = Dislike slightly
3 = Dislike moderately
2 = Dislike very much
1 = Dislike extremely

	Score based on overall liking								
Sample#	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

COMMENTS:

Once you have completed scoring each sample please lift the door to receive your free Aggie Ice Cream coupon. Thank you for participating!

Figure A2. Sample taste panel ballot: sampling and scoring section. Used for both turkey roll panel and bologna panel.

Table A3. Rotation plan for turkey roll sensory analysis. Sample numbers: 1=Control, 2=2% Sodium Lacate, 3=2% Combination of Sodium Lactate and Sodium Diacetate, 4=2% Sodium Levulinate, 5=3% Sodium Levuinate.

TURKEY	Sample Order (Sample Number- Sample Code)				
Panelist Number	1	2	3	4	5
1	2-598	5-948	4-217	3-879	1-639
2	5-829	2-491	3-943	4-467	1-796
3	4-824	1-629	5-289	2-491	3-642
4	5-743	4-457	3-358	1-275	2-259
5	1-546	2-258	4-325	5-329	3-392
6	1-741	5-745	4-894	2-856	3-631
7	5-689	3-967	1-146	2-985	4-913
8	4-578	3-749	2-269	5-364	1-219
9	1-971	4-957	5-841	3-748	2-357
10	4-543	5-268	1-923	2-658	3-716
11	3-294	5-218	2-638	1-752	4-794
12	5-287	1-523	2-652	4-716	3-912
13	5-791	4-165	3-759	1-619	2-794
14	2-952	3-965	1-362	4-358	5-256
15	1-491	5-458	3-248	2-892	4-741
16	5-697	1-389	4-893	3-129	2-456
17	1-164	2-481	3-891	5-316	4-216
18	3-284	5-653	2-457	1-964	4-479
19	5-763	1-542	2-423	4-273	3-543
20	2-312	4-869	1-241	5-579	3-946
21	2-186	4-369	5-617	3-639	1-491
22	3-381	2-987	4-347	5-534	1-856
23	4-471	2-891	1-481	3-893	5-795
24	2-879	3-129	5-478	1-639	4-239
25	5-359	1-358	4-296	3-695	2-271
26	1-847	4-685	2-517	5-732	3-412
27	5-628	1-856	2-627	3-481	4-956
28	5-894	2-629	1-576	4-251	3-814
29	5-297	4-349	1-637	3-369	2-562
30	3-652	1-648	5-471	2-659	4-186
31	4-728	1-918	3-142	2-196	5-274
32	5-851	2-214	4-892	3-752	1-174
33	5-361	2-573	3-198	4-285	1-674
34	1-893	4-142	2-758	3-867	5-524
35	5-145	2-152	4-695	3-673	1-735
36	1-897	2-138	3-152	5-982	4-631
37	1-875	4-372	5-697	3-978	2-725
38	1-745	2-325	3-241	4-697	5-971
39	3-412	5-231	2-478	1-682	4-568
40	2-856	3-823	5-217	1-539	4-794

41	1-584	5-231	3-586	4-413	2-748
42	2-835	5-157	3-794	4-482	1-671
43	4-361	1-938	2-293	5-187	3-845
44	2-195	4-653	1-172	3-164	5-729
45	4-892	1-817	3-348	2-185	5-468
46	4-521	1-831	5-482	2-943	3-642
47	3-825	5-534	2-819	1-689	4-674
48	3-629	2-641	4-153	5-134	1-386
49	5-632	4-748	1-852	3-564	2-912
50	3-637	5-286	2-954	4-812	1-942
51	2-412	4-413	1-257	5-763	3-917
52	5-287	3-195	2-942	1-421	4-814
53	5-253	4-587	1-169	3-791	2-675
54	1-182	5-142	4-785	2-324	3-745
55	4-846	5-826	1-476	3-183	2-346
56	1-963	2-586	3-429	4-165	5-384
57	2-175	1-734	5-276	4-643	3-293
58	3-291	4-674	5-893	1-821	2-637
59	3-158	4-621	5-631	2-142	1-463
60	3-928	5-153	1-853	4-965	2-672
61	1-974	2-248	3-213	5-619	4-628
62	4-249	2-857	5-143	1-527	3-418
63	1-658	3-391	5-382	4-426	2-564
64	4-653	3-682	1-984	2-198	5-589
65	5-459	3-562	1-876	2-346	4-436
66	1-594	3-685	4-492	2-314	5-264
67	3-647	4-251	1-672	5-634	2-356
68	2-842	4-697	1-754	3-745	5-489
69	3-398	5-492	1-541	2-193	4-651
70	3-324	2-374	1-982	4-534	5-752
71	4-824	2-657	5-163	1-692	3-975
72	5-836	2-149	4-795	3-391	1-289
73	2-367	3-356	1-159	5-426	4-879
74	5-165	1-865	4-467	2-291	3-245
75	1-192	3-829	5-218	4-238	2-582
76	1-693	3-487	5-739	4-736	2-268
77	5-524	2-732	1-195	4-632	3-714
78	2-453	4-325	5-578	3-389	1-768
79	2-247	3-356	5-825	4-976	1-976
80	2-281	5-924	3-712	4-392	1-126
81	2-387	4-457	5-982	3-346	1-429
82	3-214	2-645	4-578	5-471	1-734
83	3-561	2-789	1-523	4-263	5-742
84	3-367	2-524	5-846	4-263	1-569
85	5-936	4-739	2-537	1-914	3-432
86	2-397	4-715	1-386	3-683	5-415

87	4-371	3-695	2-637	5-154	1-983
88	2-983	4-197	1-937	3-864	5-176
89	5-461	3-247	1-365	2-364	4-176
90	1-692	3-539	2-648	4-347	5-957
91	2-854	5-728	3-516	1-473	4-738
92	1-341	4-784	3-682	2-495	5-326
93	3-368	5-871	4-893	2-137	1-739
94	5-815	3-162	2-173	1-546	4-368
95	2-679	3-758	1-473	5-467	4-836
96	5-872	4-847	1-754	2-432	3-258
97	1-316	3-843	2-593	5-847	4-297
98	4-519	1-785	3-789	2-428	5-968
99	2-179	3-485	5-753	1-571	1-489
100	4-531	5-978	1-264	3-672	2-681
101	2-692	3-235	4-759	1-825	5-395
102	3-937	1-341	4-924	2-583	5-861
103	3-928	5-687	2-291	1-396	1-315
104	1-437	3-843	4-759	2-279	5-847
105	5-176	3-574	1-678	2-451	4-591
106	5-142	1-654	4-834	3-149	2-258
107	4-738	3-897	2-689	5-792	1-946
108	4-673	5-627	2-318	1-925	3-985
109	5-186	2-897	1-548	3-527	4-468
110	1-286	4-361	3-256	2-916	5-671
111	5-857	2-756	3-389	4-347	1-396
112	4-351	2-572	3-539	1-742	5-521
113	5-638	2-941	4-186	3-984	1-492
114	2-536	3-918	1-317	5-879	4-726
115	1-786	2-798	3-985	4-846	5-197
116	2-391	1-613	5-273	3-839	4-382
117	1-786	4-139	5-576	3-614	2-712
118	2-475	4-518	3-614	5-517	1-146
119	4-493	5-498	1-579	2-583	3-172
120	4-823	1-798	3-418	2-734	5-562
121	4-763	1-873	3-974	2-953	5-283
122	3-781	2-137	4-326	5-371	1-276
123	4-351	2-472	5-921	1-459	3-528
124	4-893	1-254	3-941	2-418	5-369
125	1-698	3-497	2-853	5-627	4-328
126	5-529	2-453	3-931	4-142	1-594
127	2-526	3-279	4-931	5-519	1-971
128	2-147	1-327	4-615	5-478	3-487
129	2-235	5-467	3-596	4-615	1-386
130	5-723	4-173	1-968	3-247	2-832
131	4-965	3-162	5-194	2-876	1-736
132	3-432	1-783	4-541	2-672	5-531

Table A4. Rotation plan for bologna sensory analysis. Sample numbers: 1=Control, 2=2% Sodium Lacate, 3=2% Combination of Sodium Lactate and Sodium Diacetate, 4=2% Sodium Levulinate, 5=3% Sodium Levuinate.

BOLOGNA	Sample Order (Sample Number- Sample Code)				
Panelist Number	1	2	3	4	5
1	4-542	5-831	2-798	1-327	3-795
2	4-274	1-968	3-576	2-182	5-635
3	5-721	3-347	4-713	1-132	2-678
4	3-518	2-948	1-412	5-875	4-835
5	4-716	1-396	5-254	2-426	3-185
6	1-249	3-697	5-342	4-185	2-178
7	2-594	3-139	4-639	5-958	1-946
8	1-825	3-372	5-581	4-564	2-569
9	5-571	4-641	3-597	1-179	2-689
10	3-824	2-365	4-586	1-937	5-416
11	2-832	1-895	3-216	5-146	4-261
12	5-612	3-285	2-827	4-261	1-635
13	1-937	3-264	5-459	4-637	2-582
14	1-438	5-548	4-954	3-456	2-278
15	1-318	4-639	2-792	3-695	5-729
16	4-347	5-842	2-257	1-732	3-324
17	1-572	2-459	5-623	3-893	4-174
18	3-912	1-135	5-243	2-659	4-597
19	2-896	4-953	1-137	5-183	3-956
20	3-542	5-571	4-587	1-193	2-915
21	1-874	5-548	4-736	2-571	3-793
22	1-874	5-614	2-876	4-143	3-715
23	1-352	4-793	2-938	5-583	3-316
24	3-728	2-319	4-618	1-678	5-962
25	1-729	4-187	2-582	3-482	5-514
26	5-594	2-952	1-291	3-598	4-672
27	3-749	1-168	5-743	2-136	4-834
28	4-845	3-968	5-452	1-415	2-713
29	5-798	4-195	3-143	2-427	1-425
30	2-852	4-517	5-718	3-478	1-417
31	2-693	4-279	3-368	1-459	5-719
32	4-487	2-146	5-625	3-432	1-386
33	4-879	1-547	2-234	5-753	3-153
34	1-728	4-198	2-831	3-241	5-678
35	2-571	4-932	5-841	3-847	1-192
36	3-587	1-461	5-852	2-473	4-345
37	3-489	5-524	4-518	1-936	2-463
38	3-479	5-287	4-498	2-928	1-613
39	3-349	5-495	1-276	4-563	2-157
40	2-584	1-349	5-764	3-295	4-274

41	3-684	2-342	4-139	5-948	1-873
42	1-742	4-128	3-965	2-431	5-329
43	3-491	1-741	4-476	5-416	2-721
44	4-368	3-348	5-569	2-692	1-438
45	4-138	5-936	3-618	1-719	2-634
46	4-468	5-643	1-752	2-135	3-361
47	2-874	4-724	3-132	5-938	1-849
48	5-125	3-649	1-473	2-438	4-195
49	3-435	5-473	4-389	1-286	2-453
50	1-925	4-479	3-128	5-275	2-439
51	1-948	2-485	3-295	4-685	5-352
52	5-275	3-251	2-375	1-863	4-236
53	4-597	1-849	2-159	5-135	3-382
54	5-731	2-536	1-915	3-528	4-529
55	1-568	3-284	5-192	2-853	4-814
56	3-891	4-491	2-428	5-396	1-569
57	5-145	2-894	1-946	3-879	4-358
58	2-973	4-382	3-493	1-938	5-692
59	3-632	4-197	2-486	1-645	5-865
60	5-537	2-842	1-483	4-649	3-351
61	5-579	2-476	4-324	3-597	1-895
62	3-457	2-624	4-932	1-352	5-417
63	1-134	2-581	3-642	4-421	5-279
64	3-739	2-682	1-754	4-149	5-265
65	2-417	5-817	1-342	3-641	4-627
66	1-683	4-587	3-413	2-634	5-398
67	5-287	3-176	1-258	4-528	2-561
68	4-539	1-637	3-283	2-723	5-862
69	4-132	1-485	5-862	2-734	3-653
70	1-745	5-321	2-496	4-543	3-132
71	4-216	1-257	3-395	2-168	5-948
72	1-821	3-468	2-376	4-324	5-298
73	1-938	3-835	4-564	2-721	5-654
74	4-783	5-437	1-421	3-532	2-134
75	2-497	1-649	5-146	3-576	4-293
76	5-635	4-248	3-824	2-387	1-572
77	3-372	1-158	4-684	5-319	2-452
78	1-853	5-921	2-973	3-174	4-284
79	1-549	4-357	3-251	2-817	5-615
80	3-164	5-148	4-945	2-365	1-149
81	2-167	3-934	4-413	5-549	1-213
82	2-157	5-213	1-724	3-629	4-215
83	3-759	1-671	5-321	2-927	4-372
84	4-271	1-528	2-925	5-713	3-285
85	1-562	3-934	5-932	4-238	2-973
86	5-865	3-249	4-497	1-926	2-613

87	4-371	1-918	5-278	2-984	3-423
88	3-426	5-256	4-542	1-976	2-278
89	2-936	4-213	3-619	5-832	1-735
90	4-975	2-574	1-839	5-596	3-548
91	1-235	5-127	4-573	3-967	2-125
92	1-418	3-817	2-657	4-478	5-963
93	2-386	5-952	4-152	3-462	1-147
94	5-319	2-651	1-941	3-396	4-713
95	3-948	5-453	4-138	1-713	2-793
96	2-532	3-463	1-321	4-194	5-368
97	2-891	5-138	1-864	3-438	4-325
98	5-528	2-489	1-876	3-317	4-476
99	2-942	5-269	1-832	3-569	4-127
100	1-163	3-786	2-185	4-823	5-792
101	3-452	2-985	4-782	5-431	1-963
102	1-389	2-162	3-837	5-463	4-184
103	5-613	1-865	3-816	2-312	4-564
104	3-785	2-279	4-284	5-267	1-672
105	2-758	4-531	3-329	5-268	1-651
106	4-649	5-234	1-832	2-628	3-567
107	5-139	2-813	1-539	4-574	3-524
108	4-674	1-789	5-365	2-497	3-937
109	4-346	5-682	2-658	1-368	3-348
110	1-137	5-257	2-921	4-918	3-692
111	1-285	2-195	5-965	3-589	4-367
112	5-943	1-654	3-764	2-213	4-586

Appendix B

Individual Microbiological Data for Each Trial of Turkey Breast and Bologna

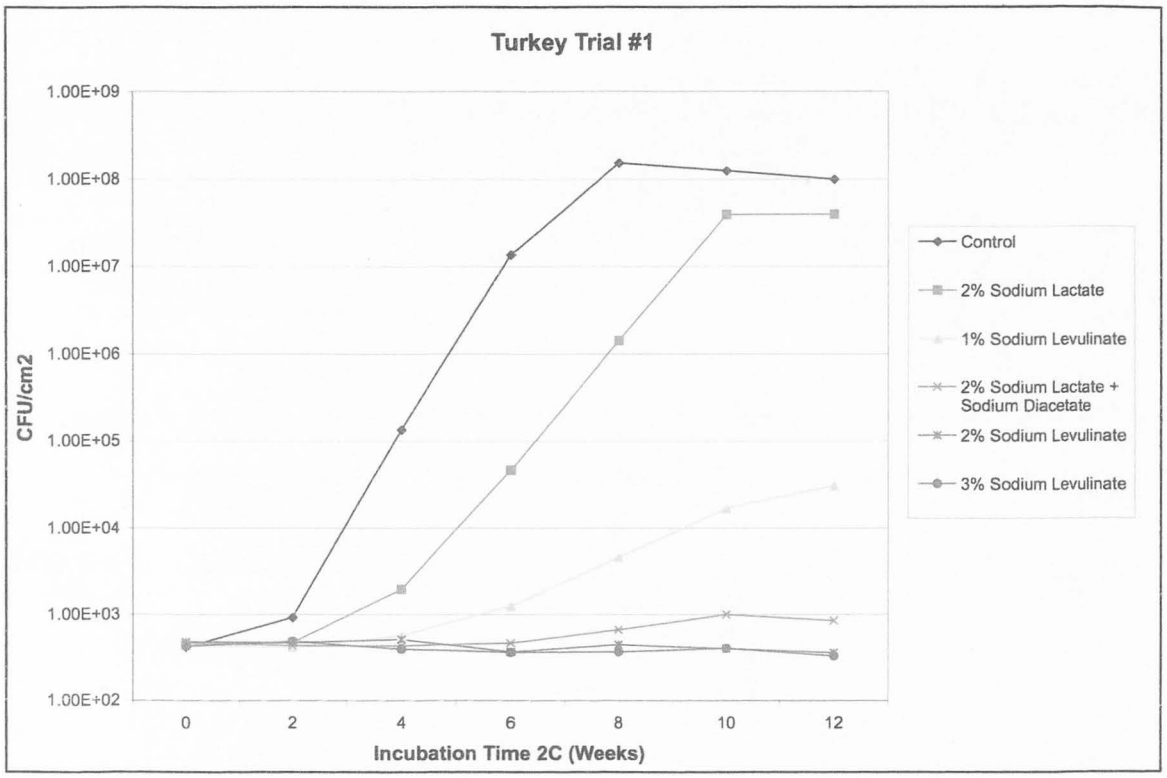

Figure B1. Trial 1 on turkey roll. Growth of *L. monocytogenes* (CFU/cm^2) during 12 weeks storage at 2°C.

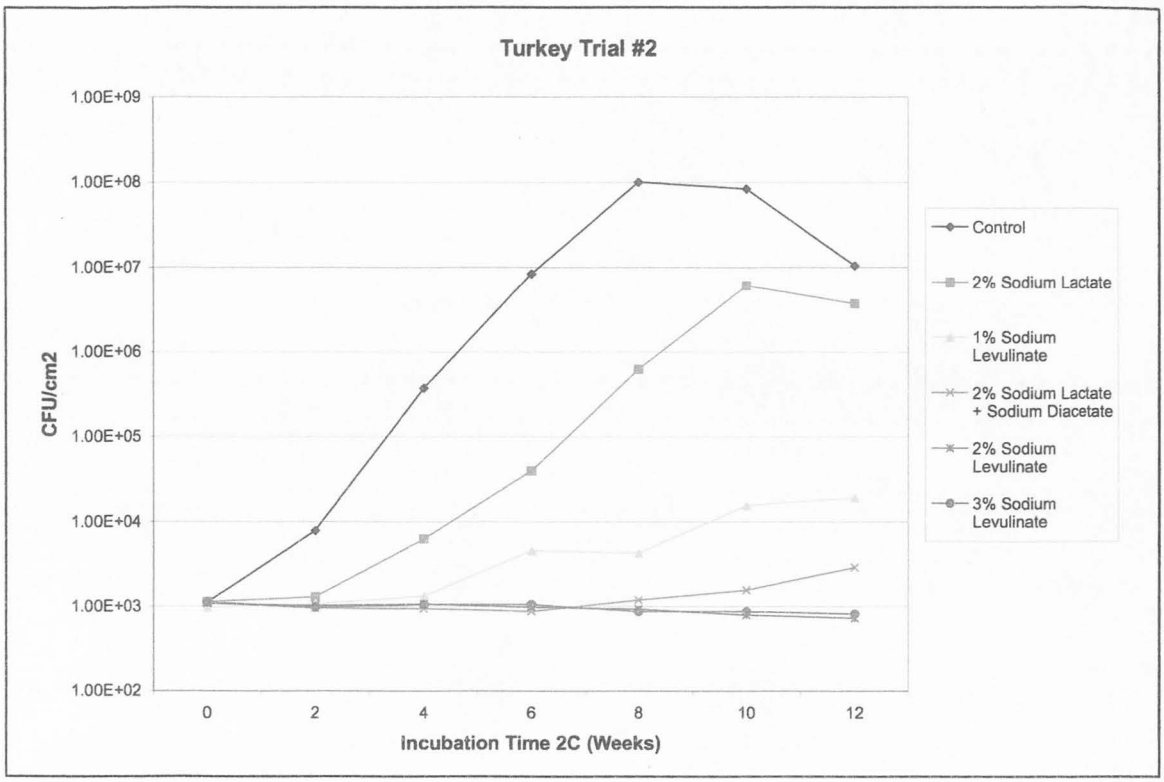

Figure B2. Trial 2 on turkey roll. Growth of *L. monocytogenes* (CFU/cm^2) during 12 weeks storage at 2°C.

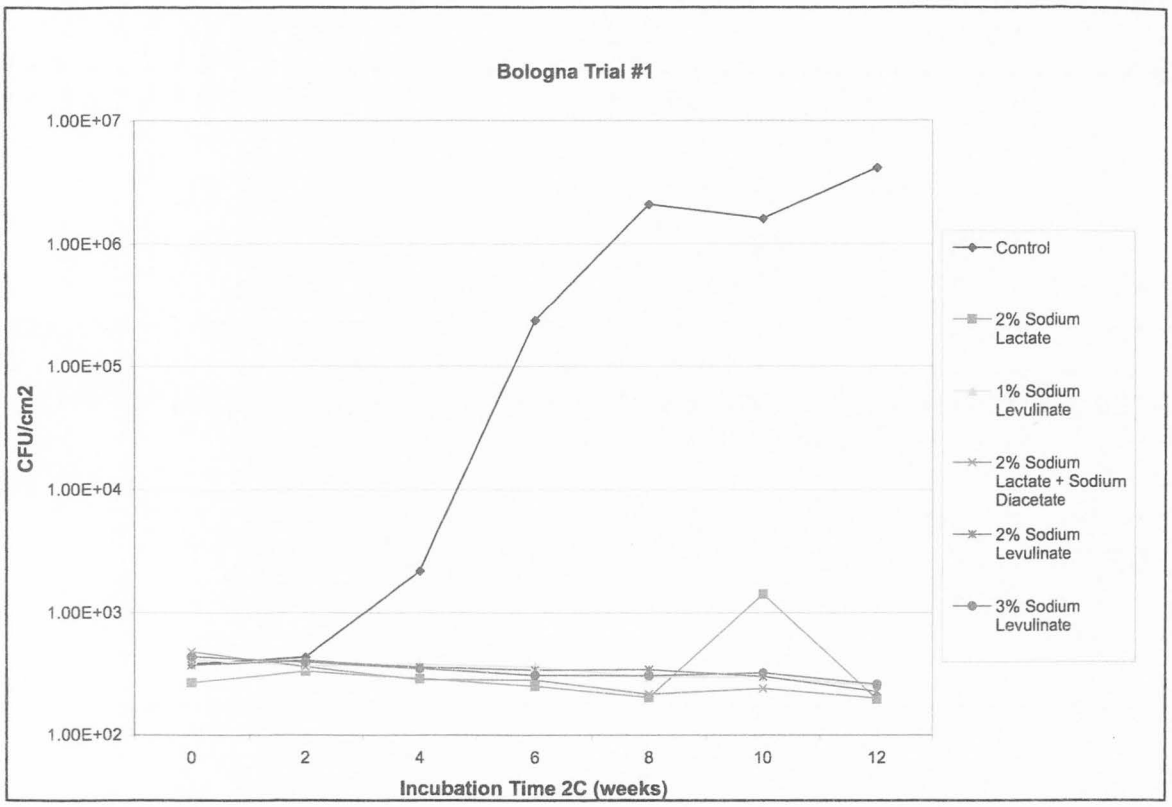

Figure B3. Trial 1 on bologna. Growth of *L. monocytogenes* (CFU/cm^2) during 12 weeks storage at 2°C.

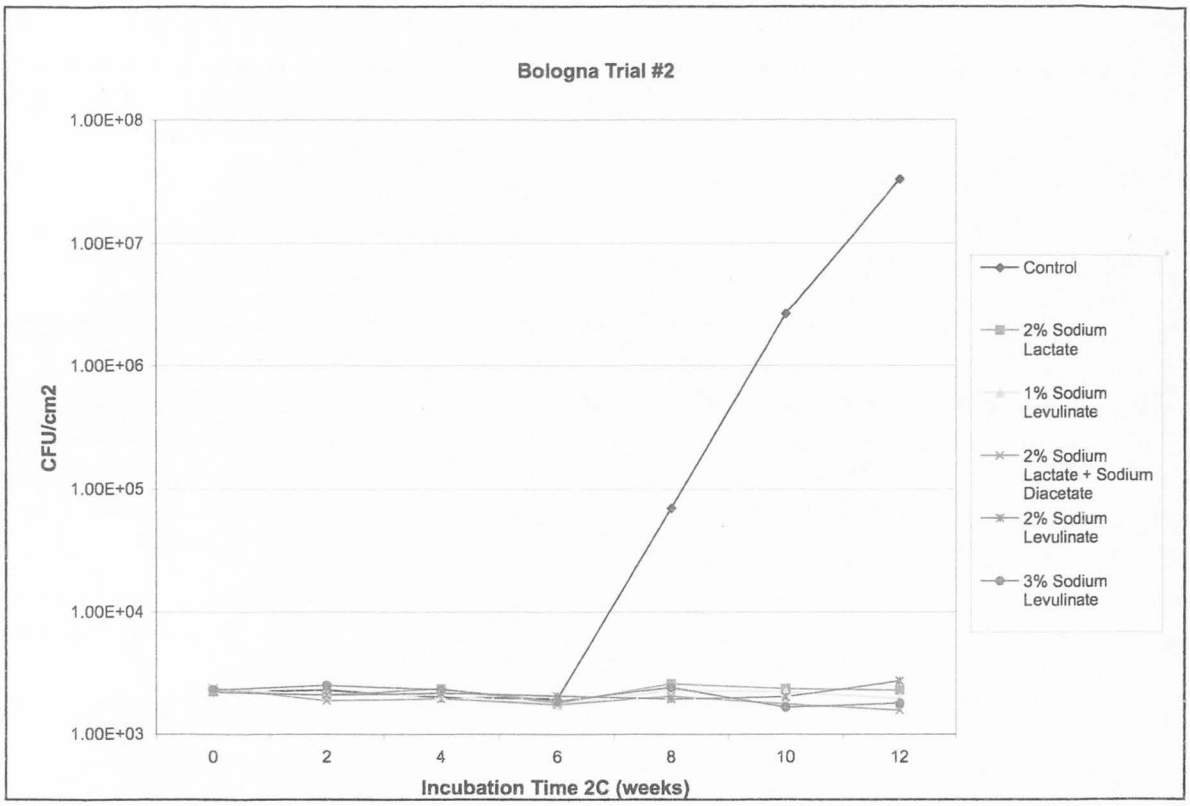

Figure B4. Trial 2 on bologna. Growth of *L. monocytogenes* (CFU/cm^2) during 12 weeks storage at 2°C.

Appendix C

ANOVA Tables for Microbiological Data

Table C1. ANOVA for microbial analysis of turkey roll.

Source	df	Mean Square	F	p-value
Treatment	5	134.52	175.06	<0.0001
Time	6	37.74	97.18	<0.0001
Treatment x Time	30	11.11	28.6	<0.0001

Table C2. ANOVA for microbial analysis of bologna.

Source	df	Mean Square	F	p-value
Treatment	5	37.55	13.22	0.0066
Time	6	4.12	6.14	0.0002
Treatment x Time	30	5.24	7.82	<0.0001

Appendix D

Comparison of Treatment Types and Adjusted p-values

Treatment Type vs Treatment Type		Adj. p-value
1	2	0.0034
1	3	<0.0001
1	4	<0.0001
1	5	<0.0001
1	6	<0.0001
2	3	0.0003
2	4	0.0013
2	5	0.0002
2	6	0.0002
3	4	0.0873
3	5	0.9343
3	6	0.9074
4	5	0.0389
4	6	0.0360
5	6	1.0000

Table D1. Comparison of treatment types on turkey roll. P-values show significance between each treatment type. 1=Control, 2=2% Sodium Lactate, 3=2% Sodium Lactate + Sodium Diacetate, 4=1% sodium Levulinate, 5=2% Sodium Levulinate, and 6=3% Sodium Levulinate.

Treatment Type vs Treatment Type		Adj. p-value
1	2	0.0105
1	3	0.0089
1	4	0.0103
1	5	0.0104
1	6	0.0104
2	3	0.9998
2	4	1.0000
2	5	1.0000
2	6	1.0000
3	4	0.9999
3	5	0.9998
3	6	0.9999
4	5	1.0000
4	6	1.0000
5	6	1.0000

Table D2. Comparison of treatment types on bologna. P-values show significance between each treatment type. 1=Control, 2=2% Sodium Lactate, 3=2% Sodium Lactate + Sodium Diacetate, 4=1% sodium Levulinate, 5=2% Sodium Levulinate, and 6=3% Sodium Levulinate.