THESIS

EFFECTS OF ANTIMICROBIALS IN THE FORMULATION AND POST-PACKAGING THERMAL TREATMENT TO CONTROL *LISTERIA MONOCYTOGENES* IN POST-PROCESSING INOCULATED FRANKFURTERS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY GERARD KOUADIO BEDIE ENTITLED "EFFECTS OF ANTIMICROBIALS IN THE FORMULATION AND POST-PACKAGING THERMAL TREATMENT TO CONTROL *LISTERIA MONOCYTOGENES* IN POST-PROCESSING INOCULATED FRANKFURTERS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

EFFECTS OF ANTIMICROBIALS IN THE FORMULATION AND POST-PACKAGING THERMAL TREATMENT TO CONTROL LISTERIA MONOCYTOGENES IN POST-PROCESSING INOCULATED FRANKFURTERS

Listeria monocytogenes presents a major food safety concern due to its ability to survive or grow at refrigeration temperatures (4°C) and in other adverse conditions (salt, heat, low pH, and large temperature range). Since *L. monocytogenes* transmission, in the case of human outbreaks, is generally associated with post-processing contamination of ready-to-eat foods, actions to eliminate or reduce the pathogen's occurrence in foods should be applied during processing and post-processing. The objectives of these studies were to determine the effectiveness of certain antimicrobials (i.e., sodium lactate at 3% or 6%, sodium diacetate at 0.25% or 0.50%, sodium acetate at 0.25% or 0.50%, nisin as Nisaplin® at 0.15%, lysozyme at 0.01%, monolaurin at 0.01%, glucono-delta-lactone at 0.25%, and allyl isothiocyanate at 0.02%, in the first study-Chapter III) or their combinations (i.e., sodium lactate at 3% with sodium acetate at 0.25%, sodium lactate at 3% with sodium diacetate at 0.25%, or sodium lactate at 3% with glucono-delta-lactone at 0.25%, in the second study-Chapter IV) included in the formulation of frankfurters against *L. monocytogenes* inoculated on their surface after peeling and before vacuum packaging, as well as the antimicrobial effect ofthermal treatment (i. e., dipping in hot water of 75-80°C for 30-90

sec; in the second study-Chapter IV). Samples were stored (for 50 or 120 days) at 4°C and periodically analyzed for microbial growth (TSAYE and PALCAM agar), pH, moisture, fat and water activity. In the first experiment of the first study (Chapter III), growth of *L. monocytogenes* reached 10^8 cfu/cm^2 in the frankfurters containing no antimicrobials during storage. Sodium lactate at 6% and sodium diacetate at 0.50% incorporated in the formulation of frankfurters singly, inhibited growth of *L. monocytogenes* stored at 4°C for 120 days. Sodium lactate at 3% inhibited growth of the pathogen for 50 days, while nisin at 0.15% added in the formulation of the frankfurters, in the second experiment, inhibited growth of the organism for 10 days. Under the conditions of the second experient in the first study (Chapter III), other antimicrobials used singly in the product did not affect growth of *L. monocytogenes* during storage. The water activity of products containing sodium acetate (0.25 or 0.50%) and sodium diacetate (0.25 or 0.50%) was slightly below that of the control (0.972) while sodium lactate decreased remarkably the water activity of the product as its concentration increased from 3% to 6% reaching, respectively, 0.946 and 0.933. Nisin (0.15%), allyl isothiocyanate (0.02)%, lysozyme (0.01)%, monolaurin (0.01)%, and glucono-delta-lactone (0.25%) did not affect the water activity of the product. None of the individual antimicrobials added in the formulation affected the cooking yield, or the moisture and fat contents of the products. On day 0, pH of the products containing sodium diacetate (0.25 or 0.50%) decreased to 6.03 and 5.87, respectively, compared to that of the inoculated control (6.31). However, pH of the inoculated control decreased dramatically to reach 5.42 during storage as growth of the pathogen reached higher levels (10^7-10^8 cfu/cm^2). Combinations of sodium lactate (3%) with sodium acetate (0.25%), sodium diacetate (0.25%), or glucono-delta-lactone (0.25%) inhibited growth of *L.*

monocytogenes for 120 days on frankfurters stored at 4°C, while growth of *L. monocytogenes* on the inoculated control frankfurters reached 10^7cfu/cm^2 in 35 days. When thermal treatment and antimicrobial combinations were applied to the frankfurters (Chapter IV), inhibition of *L. monocytogenes* was enhanced. Thermal treatment used alone on the vacuum packaged frankfurters inhibited growth of the pathogen for 35 days when one frankfurter per bag was dipped in hot (75 or 80°C) water for 90 sec. While the water activity and the cooking yield were not affected by the combination of antimicrobials in the products, sodium lactate (3%) combined with sodium diacetate (0.25%) decreased slightly the moisture while the fat content increased by approximatively 3%. The combination of sodium lactate (3%) with sodium diacetate (0.25%) or glucono-delta-lactone (0.25%) decreased the pH of the frankfurters. Moreover, the pH in the inoculated control decreased dramatically after 35 days of storage at 4°C and reached, at the end of the storage, 5.30 for the non-dipped frankfurters and 5.58 when they were dipped in hot water (80°C). The pH of frankfurters with no antimicrobials but dipped in hot (75-80°C) water dropped remarkably after 35 days in all treatments as a consequense of the high level of microbial growth on the product. In addition, the microbial counts on TSAYE agar were higher than those on PALCAM agar in all treatments during storage. These results indicate that use of combinations of permissible levels of antimicrobials in the formulation exhibited an antimicrobial activity in cooked meat products stored at 4°C for 120 days. In addition, thermal treatment may enhance the inhibitory activity of the antimicrobials during storage.

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CHAPTER I

INTRODUCTION

The ubiquity of *Listeria monocytogenes* and its ability to grow under refrigerated temperature make the pathogen of serious concern as a threat to public health. As small (0.5 μm in diameter and 1-2 μm in length) non-spore-forming gram-positive rods with rounded ends, *Listeria* cells rearrange themselves in short chains, "V" or "Y" forms, or they may be found singly in the environment. In addition, the motility of the bacterium is expressed by its peritrichous flagella when cultured at 20-35°C (Ryser and Marth, 1999). Traditionally, listeriosis occurs both in animals and humans sporadically, which is still the case in humans, and major documented outbreaks have been associated recently with processed foods (Ryser and Marth, 1999). For instance, the Centers for Disease Control and Prevention (CDC) reported that the 1998-1999 listeriosis outbreak in 11 states of the United States was related to contaminated hot dogs and deli meats (CDC, 1998). Use of chemical antimicrobials to control *L. monocytogenes* in such products has been the subject of studies. For example, Wang and Shelef (1992) found, in their study on the behavior of *L. monocytogenes* in cod fish, that a combination of lysosyme (3 mg/ml) and 0.25 mmol/l ethylenediaminetetraacetic acid (EDTA) exhibited a synergistic effect in inhibiting *L. monocytogenes* inoculated (10^3 cells/g) on the surface of the fish for 72

hours at 20°C compared to when they were used singly (approximately 12 and 18h, respectively, for EDTA and lysozyme). They reported also that when the fish was stored at 5°C, lysosyme (3mg/ml), EDTA (0.25 mmol/l) or their combination, as above, inhibited listerial growth in the product for 18 days. In another study, Qvist et al. (1994) found that growth of *L. monocytogenes* was suppressed in sausage formulated with a combination of sodium lactate (2%) and glucono-delta-lactone (0.25 or 0.50%) during storage at 5 or 10°C for 35 days. However off-flavor could be detected in the high glucono-delta-lactone product. Sodium acetate (0.50%) used in the formulation of turkey bologna (Wederquist et al., 1995) was highly inhibitory against *L. monocytogenes* for 28 days at 4°C. Blom et al. (1997) showed that the combination of sodium lactate (2.5%) and sodium acetate (0.25%) increased the shelf life of sliced and spreadable vacuum packaged sausage and cooked ham stored at 4°C for 4-6 weeks.

Several studies have evaluated food antimicrobials in the formulation of sausages, cooked meats or other ready-to-eat meat products, or in broth indicating promising results in inhibiting *L. monocytogenes*.

However, the flavor of the finished meat products was altered and the safety of the product did not last for long term storage. In order to reduce or eliminate the public health threat from *L. monocytogenes* in cooked meat products, an extended shelf life of the products should be assured. In addition, the flavor and the organoleptic quality of the products should be maintained during the shelf life. With the approval (on March 20, 2000) by the Food Safety and Inspection Service (FSIS) of petitions regarding the increase of the levels of sodium diacetate, sodium acetate, and sodium lactate to 0.25, 0.25, and 4.8% (of 60% solution), respectively, in meat and poultry products on the basis

of weight of total formulation, it would be interesting to determine if higher concentrations of these additives or their combinations may control more efficiently post-processing contamination of *L. monocytogenes* in cooked meat products such as frankfurters.

The objectives of this study were to evaluate the effectivness of higher concentrations of these antimicrobials, or their combinations, to control post-processing contamination of *L. monocytogenes* on cooked pork frankfurters. A post-packaging thermal pasteurization treatment of the products was also evaluated for its effect on the pathogen. More specifically, higher concentrations than permissible levels of antimicrobials in meat and poultry products, or combinations of permissible levels of these antimicrobials, may inhibit completely growth of inoculated *L. monocytogenes* on vacuum packaged frankfurters stored at 4°C.

CHAPTER II

LITERATURE REVIEW

Importance of *Listeria monocytogenes* in food

Listeriosis is an infrequent disease that is caused by consumption of food contaminated with *Listeria monocytogenes*. Once ingested, the pathogen reaches tissues and cells throughout the bloodstream, after colonization of the intestinal tract, and multiplies in the cytoplasm of these cells. New *L. monocytogenes* cells move to other cells by means of actin filaments forming a tail at one pole of the microorganism (Ryser and Marth, 1999). As observed in many outbreaks, the occurrence of the disease is sporadic but in 20 to 30% of the cases, it is deadly (Ryser and Marth, 1999). According to a Centers for Disease Control and Prevention report, the disease causes some 2,500 illnesses and 500 deaths annually in the United States (CDC, 2001a).

The natural environment is generally the initial habitat for virulent strains of *L. monocytogenes*. Sporadic contamination from the environment occurs in the food chain but most of the contamination occurs during food processing as the product is exposed to contamination from the plant environment (Richmond, 1990). As the minimum infectious dose is not clearly known, the Food and Drug Administration (FDA) and the FSIS have set zero tolerance for *L. monocytogenes* in ready-to-eat products (FSIS, 2000).

The symptoms of listeriosis are many and depend on the state of the host (Jay, 1996). People susceptible to the disease are generally immunocompromised, elders, pregnant women, and newborns. A CDC study associated illness with consumption of Mexican style cheese, feta cheese, delicatessen foods, other ready-to-eat foods, and undercooked chicken (CDC, 2001b). Symptoms include spontaneous abortion in pregnant women, meningitis in newborn infants, prenatal septicaemia, meningocephalitis in adults, fever, muscle aches, nausea, and diarrhea (Al-Sheddy et al., 1995). Moreover, if the infection spreads to the nervous system, headache, stiff neck, confusion, loss of balance, or convulsions can occur (Jay, 1996). The fatality rate of listeriosis is approximately 30% in immunocompromised, newborn, elderly, and young people (Al-Sheddy et al., 1995). *Listeria* is a gram-positive, nonsporeforming bacterium that was classified once as *Listerella,* but changed in 1940 to *Listeria* (Jay, 1996). The same author reported that the bacterium is similar to *Brochrotrix* regarding the ability to hydrolyze hydrogen peroxide (catalase positive). Both *Listeria* and *Brochrotrix* are found in nature in association with *Lactobacillus* (Jay, 1996). All three genera produce lactic acid as they ferment glucose and other sugar substrates, but *L. monocytogenes* is xylose negative, mannitol negative and rhamnose positive. According to the same author, six species of *Listeria* (*L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii, and L. grayi*) are currently recognized. The most important one is *L. monocytogenes* because it is the only human pathogenic species (Jay, 1996).

As a gram-positive bacterium, *L. monocytogenes* grows in many common media such as brain heart infusion (BHI), trypticase soy agar (TSA), and tryptose broths. Growth of this pathogen requires at least three B vitamins, including biotin, riboflavin, thiamin, as well

as thioctic acid (an alpha lipoic acid used as a growth factor by some bacteria and protozoa), and the amino acids cysteine, glutamine, isoleucine, leucine, and valine (Jay, 1996). As reported by Ryser and Marth (1999) the pH range for growth of *L. monocytogenes* is 4.1 to 9.6 with an optimum of pH 7. The organism grows in the temperature range of 1 to 45°C with an optimum of 30 to 37°C. The minimum water activity required for the growth of the pathogen is around 0.90-0.92 with an optimum of 0.97. Studies performed by Cole et al. (1990) showed that *L. monocytogenes* was able to grow in 4.0% to 6.0% NaCl solution within 13 to 28 days at pH 4.66 and at 30°C. The ability of *L. monocytogenes* to grow well under both aerobic and anaerobic conditions as well as under refrigerated temperatures represents a potential threat to the safety of vacuum packaged foods (Ryser and Marth, 1999). In a recent study, top round beef patties (< 4% fat) inoculated (3.58-4.67 cfu/g) with four strains of *L. monocytogenes* (N-7143, Scott A, Na-16, and Na-19), vacuum packaged and stored at 4°C, indicated growth of all strains except for Scott A at pH 5.47 and 6.14 for 42 days (Barbosa et al., 1995). It was concluded that growth of *L. monocytogenes* increased rapidly at pH above 6.0 and depended on the strain of the pathogen. Although growth of strain Scott A was low in the study above, the results cannot be applied to *L. monocytogenes* in general since it may be found in the environment or in foods with a mixture of more than four strains. Research on the influence of growth temperature (4, 20, and 37°C) on thermal (62°C) resistance of *L. monocytogenes* in a thermoresistometer indicated that the maximum heat resistance of the pathogen was greater and was reached more rapidly at higher growth temperatures (Pagan et al., 1998). In addition, the authors reported that the maximum D_{62}- values of cells grown at 4, 20, and 37°C were 0.13, 0.18, and 0.34 min, respectively, indicating an

increase of the heat resistance as the incubation temperature increased. Ryser and Marth (1999) reported that, *L. monocytogenes* survived in Cheddar cheese of water activity between 0.972 and 0.979 at pH 5.0-5.1 for 224 and

more than 434 days when the product was stored, respectively, at 13 and 6°C.

Occurrence of *Listeria monocytogenes* in food

In the last decade research has shown that *L. monocytogenes* can be found anywhere and in any type of food. The environmental origin of this pathogen allows detection of contamination in products of plant origin. Even though cases of listeriosis in plant products are not as common as in other types of food, the 1981 listeriosis outbreak in Canada, where 18 of 41 poeple infected with *L. monocytogenes* died was associated with consumption of coleslaw (Ryser and Marth, 1999). The authors reported that one isolated case of listeriosis was reported in Finland from contaminated home-made salted mushrooms. In Texas, five cases of listeriosis were related to consumption of broccoli and cauliflower (Simpson, 1996). *Listeria monocytogenes* has been detected in other types of foods as confirmed by the 1985 listeriosis outbreak in southern California involving consumption of Mexican-style cheese (Ryser and Marth, 1999) where of 300 cases 40 died. Tests performed in food processing facilities by the FDA officials in cold-smoked fish plants resulted in detection of *L. monocytogenes* on processing materials from thawing water in the raw product and processing area to product trimmings from slicers in the finished product and processing area (Ryser and Marth, 1999).

Occurrence of *Listeria monocytogenes* in meat products

In the last decade, cases of listeriosis have been associated with comsumption of meat or meat products such as sausages and frankfurters. Results from the FSIS *Listeria* sampling programs showed that 4.4% of 4980 samples of small diameter sausages evaluated were found positive for *L. monocytogenes* (FSIS, 1999). Results from the same FSIS program in ready-to-eat meat products including beef jerky, cooked sausage (large diameter, and small diameter) concluded that small-diameter cooked sausage contained higher prevalence of *L. monocytogenes* than large-diameter cooked sausage. Of 8000 ready-to-eat meat samples tested in Northern Ireland, 5% were found to contain *Listeria* species, of which 49% were *L. monocytogenes* (Wilson, 1995). In North America an 8% incidence of *L. monocytogenes* was found in 19 brands of retail wieners (Wang and Muriana, 1994). A study in Canada reported that from 38 samples of wieners and 29 samples of sliced meats, 21 and 13.8%, respectively, were contaminated with *L. monocytogenes*, while of 38 packaged luncheon meat samples tested 13.1 % were found positive for the pathogen (Tiwari and Aldenrath, 1990). In Europe and other countries contamination of sausages or ready-to-eat products was also reported (Ryser and Marth, 1999).

Effect of thermal treatment on *Listeria monocytogenes*

Based on studies reported by Lovett et al. (1990), it was concluded that pasteurization is a safe process reducing the number of *L. monocytogenes* occurring in raw milk to levels that are not considered risky for human health. Furthermore, investigation of the effect of meat curing agents on thermal destruction of *L. monocytogenes* in ground pork indicated

that the greatest protective effect on thermal destruction (60°C in a waterbath) was observed when curing ingredients such as sodium chloride, dextrose, phosphate mixture, sodium nitrite, and sodium erythorbate were combined and added to ground pork (Yen et al., 1991). Thermal inactivation (microwave heating) of *L. monocytogenes* on chicken skin showed that when a temperature of 70°C was reached and maintained for at least 2 min throughout the food, there was substantial reduction (10^6 to 10^8 cells) in the number of *L. monocytogenes* (Coote et al., 1991). Bradshaw et al. (1991) reported that resistance of *Listeria* spp. other than *L. monocytogenes* to high temperature short time (HTST) treatment was equivalent to that of *L. monocytogenes* in raw and sterile milk. Another HTST (72-73°C for 15-16sec treatment) study conducted by Lovett et al. (1991) on raw milk containing 10^5 cfu/ml indicated that no *L. monocytogenes* survived the treatment, when using proper equipment and procedures. On the other hand, sodium chloride (2.0 to 2.5%) alone or in combination with dextrose (1%), sodium erythorbate (0.055%, sodium nitrite (0.0156%) added to ground beef heated at temperatures of 50 to 60°C reduced the extent of thermal destruction of *L. monocytogenes* but not when the temperature exceed 67°C (Yen et al., 1992). Even though reduction of 4.4 to 6.1 log of *L. monocytogenes* was observed from the inoculated level of 8.08 log cfu/g in ground beef heated at 50, 60, and 65°C, growth was found when samples were cultured after 21days in selective enrichment medium (24h) (Boyle et al., 1990). The synergistic effect of nisin and heat on *L. monocytogenes* was demonstrated when nisin resistant *L. monocytogenes* (Nis^r) was submitted to heat (55, 60, 65°C) in 2-(N-Morpholino) ethanesulphonic acid buffer pH 7.0 in presence of or absence of nisin (Modi et al., 2000). The results of the study indicated that in presence of nisin, nis^r *L. monocytogenes* was more sensitive to heat than in the

absence of nisin. The D-values supporting these results were lower (D_{55}-values of Nis^r ATCC700301 and Nis^r ATCC700302, 2.88 and 2.77min), compared to when there was no nisin, (3.86 and 3.83min, respectively). However, D-values were not significantly different at 65°C between the Nis^r cells and the wild type *L. monocytogenes* at 65°C regardless of the presence or the absence of nisin.

Effect of chemical additives on *Listeria monocytogenes*

Sodium acetate. Sodium acetate (0.5%) incorporated in the formulation, significantly decreased growth of *L. monocytogenes* for 70 days at 4°C, more than sodium lactate (2%) and potassium sorbate (0.26%), in refrigerated vacuum packaged turkey bologna (Wederquist et al., 1994). Sodium acetate was the most inhibitory against *L. monocytogenes* at that level, followed by sodium lactate (2.0%) and potassium sorbate (0.26%) according to the same studies. Schmidt (1995), reported that, when sodium acetate (1%) was added to a sausage mixture contaminated with lactic acid bacteria (10^5 lactic acid bacteria/g), growth of inoculated *L. monocytogenes* was inhibited without lactic acid bacteria spoiling the sensory quality of the sausage mixture stored at 5°C for 3 weeks. Research conducted by Wederquist et al. (1995) on surface inoculated turkey bologna containing varying additives indicated that sodium acetate (5g/kg) at pH 6.63 was highly inhibitory to *L. monocytogenes* for 28 days of storage at 4°C. Fresh shrimp and catfish filets pieces dipped separatly in sodium acetate (2%) for 30 min and overwrapped in polyvinylidene chloride (PVDC) film and stored at 4°C indicated no difference in growth of psychrotrophic microorganisms on the shrimp compared to the

control whereas in the catfish, the shelf-life was extended for 3 days (Zhuang et al., 1996).

Sodium diacetate. Research with turkey slurries (Schlyter et al., 1993a) indicated that sodium diacetate (0.5%) added to turkey slurries stored at 25°C, inhibited growth of *L. monocytogenes* for 7 days. When stored at 4°C, a lower concentration (0.3%) of the chemical was needed to inhibit growth of the pathogen for 7 days. Furthermore, the authors reported that a combination of sodium diacetate (0.3 or 0.5%) with pediocin (5000 Arbitrary Units/ml) had a greater listericidal activity than other treatments (Schlyter et al., 1993b). Sodium diacetate (0.3%) added to ground beef or beef slurry and stored at 5°C resulted in complete suppression of aerobic bacteria and *L. monocytogenes* for 35 days (Shelef and Addala, 1994). Steam sterilized crab meat inoculated with *L. monocytogenes* and washed with sodium diacetate (2 mol/l) resulted in a decrease of 2.6 log cfu/g within 6 days at 4°C (Degnan et al., 1994).

Sodium lactate. Hu and Shelef (1996) reported that 1.8% sodium lactate in the formulation of a pork liver sausage, with a fat content of 22%, inoculated with 10^4 cfu/g *L. monocytogenes* exhibited a listericidal effect (-0.5 log) at 4°C for 50 days. The listericidal effect increased (-1.8 log) as the fat content increased to 67% in the pork liver. In a study evaluating the effect of sodium lactate and heat (McMahon et al., 1999), inactivation of *L. monocytogenes* was observed when minced beef containing sodium lactate (2.4 or 4.8%) was heated at 55°C and plated on recovery medium containing the same concentrations of sodium lactate. Qvist et al. (1994) reported that sodium lactate (2

%) in the formulation of a sausage, stored at 5°C, suppressed growth of *L. monocytogenes* for 28 days. In contrast, sodium lactate (1.8%) added to ground beef inoculated with *L. monocytogenes* and cooked for 20 min at 65°C showed no significant difference with the cooked minced beef (20 min for 65°C) with no additives (Harmayani et al., 1993). Weaver and Shelef (1993) reported that, sodium lactate (3%) added to a mixture of liver sausage after peeling and inoculation with *L. monocytogenes* inhibited growth of the organism for 50 days at 5°C. Moreover, 2 or 3% sodium chloride combined with 2 or 3% lactate (salt of calcium, potassium or sodium) enhanced the inhibitory effect of the lactate on growth of *L. monocytogenes*. Potassium lactate (4%) in comminuted cooked beef, inhibited growth of *L. monocytogenes* for 2 weeks when the product was stored at 5°C (Shelef and Yang, 1991). However, in the same study, addition of sodium chloride (3%) to potassium lactate (4%) did not alter the growth of the pathogen. In relation with moisture and water activity, sodium lactate (4%) suppressed growth of *L. monocytogenes* for 7 days when added to cooked meat containing a moisture content of 25 to 55% with a water activity of 0.886 to 0.964, respectively (Chen and Shelef, 1992). Sodium lactate (3.6%) injected to cold smoked fish and stored at 8°C prevented growth of *L. monocytogenes* for 17 days while when injected to the fish before smoking and stored at 3°C, growth of the organism was inhibited for about 15 days (Nykanen et al., 2000).

Glucono-delta-lactone. The literature does not contain many studies on glucono-delta-lactone applied to meat products in order to control growth of *L. monocytogenes*. However, in a study using two levels of glucono-delta-lactone (0.25% and 0.5%) combined with 2.0% sodium lactate in the formulation of a bologna-type sausage in order

to test their effect on *L. monocytogenes* inoculated on the surface of bologna slices, the results showed that growth did not occur within 35 days at 5°C or 10°C (Qvist et al., 1994). A D-value determination study was performed on mussel marinades containing different chemicals and exposed to seven strains of *L. monocytogenes* (10^6 cfu/g) and stored at 5°C (Bremer and Osborne, 1995) indicated that the marinades containing 1.5% acetic acid and 0.2% glucono-delta-lactone resulted in a lower D_5-value (19.3 h) compared to marinade containing acetic acid (0.75%) and lactic acid (0.75%).

Nisin. A sorbate (0.2%)-nisin (40 IU/ml) combination applied on the surface of beef steaks and stored at 4°C resulted in inhibition of *L. monocytogenes* for 4 weeks (Avery and Buncic, 1997). Recently, a study of the combination of nisin (5.3 μg/ml) and 10 mmol/l of carvacrol (oil of oregano), in a broth containing *L. monocytogenes* showed a reduction of bacterial counts greater than when nisin was used alone, attesting the synergistic effect of both compounds, while adding lysozyme to the combination, enhanced this synergistic effect (Pol and Smid, 1999). Buncic et al. (1995) tested the effect of combinations of sodium lactate (4%) and nisin (400 IU/ml), on *L. monocytogenes* in Brain Heart Infusion (BHI) broth at 4°C. They concluded that the combination decreased the number of *L. monocytogenes* in the broth by 2.2-2.4 logs when stored at 4°C for 4 weeks. The effect of the antimicrobials in BHI was bactericidal against *L monocytogenes* but in a meat product matrix it can be different because of differences in substrate. In another study, slices of smoked salmon inoculated with *L. monocytogenes*, surface treated with nisin (400 IU/g and 1250 IU/g) and stored at 4 or 10°C indicated a failure of nisin to prevent growth of the pathogen (Szabo and Cahill,

1999). Raw beef inoculated with *L. monocytogenes* (5.33 log), immersed in nisin solution (0.04%) and stored at 4°C, showed inhibition of the pathogen for 42 days. When nisin was combined with lactic acid (2%) or polylactic acid (2%), the antilisterial effect was even more accentuated during storage (Ariyapitipun et al., 2000). Inoculated (10^5 cfu of *L. monocytogenes* /g) cold-pack canned lobster containing nisin (0.25%) and heated at 60°C for 5 min or 65°C for 2 min resulted in reduction of the pathogen by 3 and 5 logs, respectively, while nisin alone caused a reduction of 3 logs (Budu-Amoako et al., 1998). Nisin (500 or 1000 IU/ml) added to cold smoked salmon inoculated with *L. monocytogenes* and stored at 5°C failed to prevent growth of the organism, althought it was delayed, but when in addition to nisin the product was packed in 100% CO_2, the pathogen was reduced to below the inoculated level (10^3 cfu/g) during 27 days (Nilsson et al., 1997). Fresh meat slurry inoculated with *L. monocytogenes* (10^6 cfu/ml) and treated with nisin (2000 IU/ml) exhibited a listeriostatic effect for 24 h at 20°C while a listericidal effect was shown as nisin was combined with lactocin 705 and enterocin CRL35 in 24 h (Vignolo et al., 2000). Pawar et al. (2000) investigated the effect of the combination of nisin (800 IU/g) and sodium chloride (2%) in buffalo minced meat inoculated with *L. monocytogenes* (10^3 cfu/g) and stored at 4°C. The results indicated that the combination inhibited growth of the microorganism for 16 days.

Monolaurin. Monolaurin tested in trypsic soy broth with yeast extract (TSBYE) at pH 5.0, 5.5, 6.0 and 7.0 at 35°C for 24 h against *L. monocytogenes* showed a reduction of the minimum inhibitory concentration from 10 to 3 μg/ml when the pH decreased from 7.0 to 5.0, indicating an icreasing sensitivity of *L. monocytogenes* to monolaurin as the pH

decreased (Oh and Marshall, 1992). Different concentrations (5, 6, 7, 8 and 9μg/ml) of monolaurin were tested in TSBYE against *L. monocytogenes* at pH 5.0, 5.5 and 7.0, and at 7, 15 and 35°C. The results indicated the effectiveness of monolaurin at all concentrations at pH 5.0 when the medium was incubated at 7°C. It was also concluded that the inhibition of the organism by monolaurin increased as the incubation temperature decreased at constant pH (Oh and Marshall, 1993). Research on the influence of packaging methods, monolaurin (200μg/ml) and lactic acid (0.5%) on the growth of *L. monocytogenes* in sterilized crawfish tail meat dipped in 2 liters of 10^5cfu/ml of the inoculum led to an inhibitory effect of the combination by reducing the growth of *L. monocytogenes* to approximately 10^5 cfu/g compared to the control (10^7 cfu/g) when the product was storaed at 4°C for 14 days (Oh and Marshall, 1995). Other combinations of monolaurin (250 ppm), eugenol (500 ppm), and sodium citrate (0.2 or 0.4%) in TSB or MRS broth (pH 6.5 + 2% NaCl) stored at 7°C, suppressed growth of *L. monocytogenes* (10^7 cfu/ml) after 4 days (Blaszyk and Holley, 1998). Combination of monolaurin (0.72 mmol/l) with lactic acid (56, 112 and 224 mmol/l) added to crawfish tail meat mixture inoculated with *L. monocytogenes* (10^5 cfu/ml) and stored at 4°C indicated an increase in the inactivation of the pathogen as the concentration of lactic acid increased (Oh and Marshall, 1994). The same authors reported that, the number of *L. monocytogenes* dropped below the detection level at concentrations of 0.72 mmol/l monolaurin and 224 mmol/l lactic acid after 10 days when the crawfish tail meat mixture was stored at 4°C (Oh and Marshall, 1994). Investigation of the effect of spreading monolaurin solution (200 μg/cm^2) on an Italian cheese (stracchino cheese) dipped in 800 ml *L. monocytogenes*

suspension (5 x 10^4 cfu/ml) resulted in a decrease of the organism count by 1.5 logs during storage at 5°C for 12 days (Stecchini et al., 1996).

Lysozyme. *Listeria monocytogenes* was inhibited by lysozyme (1%) in phosphate buffer for 6 h at 37°C (Hughey and Johnson, 1987). Another study, demonstrated the inhibition of *L. monocytogenes* by egg-white lysozyme (10μg/ml) in trypsic soy broth at 5°C for 60 days when the pH was lowered from 7.2 to 5.5 (Johansen et al., 1994). Gill and Holley (2000) evaluated the effect of the combination of lysozyme, nisin and ethylenediaminetetraacetic acid (EDTA). It was found that the combination of the 3 compounds inhibited *L. monocytogenes* inoculated (dipped in an inoculum suspension of 4 logs/cm^2 of the pathogen) on ham and bologna (coated in gel containing 0.6% lysozyme, 430 ppm nisin and 2.55% EDTA) for 3 and 4 weeks, respectively, when the products were stored at 4°C. A previous study of the same authors indicated that a combination of lysozyme-nisin (1:3) (500 mg/kg), and EDTA (500 mg/kg) added to the formulation of vacuum packed sausages resulted in inhibition of *L. monocytogenes* (10^4 CFU/cm2) for two weeks when the sausages were stored at 4°C (Gill and Holley, 2000). After demineralization (Ca^{2+} and Mg^{2+}) of milk, lysozyme (10% w/v) inhibited growth of *L. monocytogenes* for 6 days at 4°C (Kihm et al., 1994). Moreover, when heat (55°C for 5 min) was applied to the demineralized milk, a decrease of 6 logs in the pathogen counts was observed.

According to Hughey and Johnson (1987), the lysis of several pathogenic bacteria, including *L. monocytogenes*, was enhanced when lysozyme was used in combination with EDTA, which may bind a necessary metal ion indispensable for the growth of the

organism or may disrupt the outer cell wall structure allowing lysozyme to penetrate to the peptidoglycan.

Allyl isothiocyanate. Work on allyl isothiocyanate incorporation in the formulation of foods has been limited compared to other antimicrobials. However, an investigation of the antimicrobial effect of allyl isothiocyanate (AITC) and methyl isothiocyanate (MITC) on *L. monocytogenes* inoculated on iceberg lettuce (10^4 cfu/g) kept at 4°C, showed that vapor of 400 µl of the antimicrobials inhibited the organism for 4 and 2 days, respectively, but failed when the inoculum level was at 10^8 cfu/g (Lin et al., 2000b). Another study applying dissolved AITC to *L. monocytogenes* (Lin et al., 2000a) indicated that a concentration of AITC, as much as 2500 µg/ml, was able to inhibit growth but was not listericidal in Butterfield's phosphate buffer (BPB) after 3 h at 37°C. Vapor generated from 400 µl AITC was used in the same study against *E. coli* O157:H7 (10^4 cfu/g). The results indicated no detectable *E. coli* O157:H7 after 24 h on the product. In a recent study, *L. monocytogenes* inoculated on the surface of agar plates (10^0 to 10^6 cfu/cm^2) and incubated in close jars containing gaseous AITC (500, 1000, 1500 and 2000µg/liter of air) indicated inhibition of the pathogen by AITC concentrations greater than 1000 µg/liter with the highest cell density at 30°C (Delaquis and Sholberg, 1997). The same author reported that AITC (500 and 1500µg/liter) inhibited growth of the organism at 5°C and 40°C, for 7 days and 48h, respectively.

Product inoculation and bacteria removal from the surface of the frankfurters

Several methods of inoculation and removal of bacteria from the surface of frankfurters were used in many studies before further analysis of the products. Research showed that inoculum was distributed along the length of frankfurters placed in plastic pouches and the frankfurters were rolled inside the pouches to spread the inoculum all over them (Berry et al., 1991). In other studies, frankfurters were inoculated by dipping them in a bacterial suspension for 30 sec, and then they were removed immediately thereafter (Palumbo and Williams, 1994; Buncic et al., 1990). Removal of the bacteria from the surface of the frankfurters was done by massaging and shaking the bags for 1 min followed by a 2 min maceration in a stomacher bag (Berry et al., 1991). In other cases, the bacteria were removed from the surface of the frankfurters simply by shaking the stomacher bag containing the frankfurters by hand (Palumbo and Williams, 1993; Buncic et al., 1990).

Among the food chemicals described above, sodium acetate, sodium diacetate, sodium lactate, and glucono-delta-lactone are the only additives approved by the FSIS for use in meat and poultry product as antimicrobials or flovoring agents. Other chemicals are used mostly in research where their antimicrobials activity is still being evaluated.

CHAPTER III

SINGLE ANTIMICROBIALS IN THE FORMULATION TO CONTROL POST-PROCESSING CONTAMINATION BY *LISTERIA MONOCYTOGENES* ON FRANKFURTERS STORED AT 4°C IN VACUUM PACKAGES

ABSTRACT

Post-processing contamination of cooked meat products by *Listeria monocytogenes* may be a serious hazard to public health. Fatal listeriosis outbreaks such as the 1998-multistate outbreak in the United States indicate the importance of *L. monocytogenes* contamination in meat products. The objective of this study was to evaluate antimicrobials in the formulation of frankfurters for the control of *L. monocytogenes* during storage at 4°C in vacuum packages. Individual batches, except for the control frankfurters, were prepared to contain sodium lactate (3% or 6%), sodium diacetate (0.25% or 0.50%), sodium acetate (0.25% or 0.50%), nisin (Nisaplin®; 0.15%), lysozyme (0.01%), monolaurin (0.01%), glucono-delta-lactone (0.25%), or allyl isothiocyanate (0.02%) as single antimicrobials in the formulation. After peeling (removal of the casing), sticks (11 cm) of frankfurters were surface inoculated (10^3-10^4 cfu/cm^2) with a cocktail of ten strains of *L. monocytogenes*, then vacuum packaged. All samples were stored at 4°C for 50 or 120 days and were periodically (0, 10, 20, 35, 50, 70, 90, and 120 days) analyzed for bacterial

counts on Tryptic Soy Agar with 6% yeast extract (TSAYE) and on PALCAM agar. Fat and moisture content, pH, and water activity were also determined. Counts on PALCAM agar reached 10^8 cfu/cm^2 in inoculated control products during storage. Sodium lactate at 6% and sodium diacetate at 0.50% inhibited growth and even decreased counts of *L. monocytogenes* during storage for 120 days, while at 3%, sodium lactate inhibited growth for 50 days at 4°C. Sodium diacetate (0.25%) and sodium acetate (0.25% and 0.50%) inhibited growth of the pathogen for 20 days. The other antimicrobials exhibited no effect on the growth of *L. monocytogenes* during storage for 50 days, except for nisin (Nisaplin®, 0.15%), which inhibited growth only for 10 days. The pH of the product was not affected, except when sodium diacetate was added at 0.50%, which reduced pH by 0.4 unit. Sodium lactate decreased the water activity of the product and the decrease was more pronounced as the concentration of the chemical increased from 3% to 6%. The cooking yield, moisture and fat contents were not affected by the antimicrobials. These results indicate that permissible level of sodium acetate or sodium diacetate inhibited growth of *L. monocytogenes* for 20 and 35 days while permissible concentration of sodium lactate inhibited growth for 50 days at 4°C. By doubling the permissible levels of these antimicrobials, total inhibition of the pathogen was observed with sodium diacetate and sodium lactate while sodium acetate inhibited growth for 35 days at 4°C.

INTRODUCTION

The concern of consumers and the food industry about the presence of *Listeria monocytogenes* in food, particularly in meat products, has increased in recent years. As a

matter of fact, many listeriosis outbreaks have occurred in several states of the United States showing infection of people via diverse food products. A recent outbreak (1998-1999) in 11 states indicated that contaminated hot dogs and deli meats were the vehicle of infection of the consumers (MMWR, 1999). Schlech (2000) reported in his study on foodborne listeriosis that hot dogs, undercooked chicken, delicatessen meats, unpasteurized cheese, and soft cheese appear to be frequently the origin of infection. He indicated also that the prevalence of *L. monocytogenes* in humans' intestinal tract is around 2-10%, and that it increases during an outbreak. The August 1994 to June 1995 listeriosis outbreak in Sweden involved post-processing contamination of vacuum-packaged cold smoked trout (Tham et al., 2000). Further investigations, indicated that one of the contaminated trout, found one week later, contained 2.5 million cfu/g and that the source of the contanination was the packaging machine during the packaging process. Another survey (July 1987 to January 1988) on refrigerated or frozen cooked crabmeat indicated contamination of *L. monocytogenes* in different countries (Elliot and Kvenberg, 2000). During this survey, 4% of 98 samples (in the United States) and 18.2% of 11 samples (in Korea) analyzed were found positive for the pathogen. Testing performed by Samelis and Metaxopoulos (1999) in meat (turkey necks and breast, pork trimmings, and lard) found contamination and survival of *L. monocytogenes* in meat cooked in broilers (below 70°C) while oven cooked (generally in a smokehouse) meat and emulsion-type sausages cooked at 72-75°C were *Listeria* free. Among the major reasons that listeriosis should be considered as a threat and should be treated seriously is the presence of *L. monocytogenes* in cooked foods due to post-processing contamination of the products and its ability to grow at refrigerated temperatures. It appears that not all consumers reheat

ready-to-eat food products before consumption. In addition, failure of applying good manufacturing practices in food service may cause contamination of the products and infection of the consumer. It is then necessary to find a way to keep the safety of the product intact until consumption.

The objective of this study was to evaluate antimicrobials incorporated in the formulation of pork frankfurters as inhibitors of growth of post-processing contamination of *L. monocytogenes* in vacuum packages at 4°C. The hypothesis that peremissible and greater levels of sodium acetate, sodium diacetate, and sodium lactate would inhibit *L. monocytogenes* was tested. The effect of nisin, lysozyme, monolaurin, glucono-delta-lactone, and allyl isothiocyanate included in the formulation at a single concentration was also evaluated.

MATERIALS AND METHODS

Culture preparation

The inoculum used in this experiment was a mixture of ten strains of *Listeria monocytogenes*, which included Scott A (serotype 4b, human isolate), NA-3 (serotype 4b, pork sausage isolate), NA-19 (serotype 3b, pork sausage isolate), LM103M (serotype 1a, pork sausage isolate), LM101M (serotype 4b, pork sausage isolate), 558 (serotype½, pork meat isolate), and PVM1, PVM2, PVM3, PVM4 (pork variety meat isolates). Tryptic Soy Broth (BBL, Becton Dickinson Co., Cockeysville, MD) was prepared with 0.6% yeast extract (Difco, Becton Dickinson Co., Sparks, MD) (TSBYE) and 10 ml portions were dispensed into clean test tubes and sterilized at 121°C for 15min. Portions of 0.1 ml of frozen stock cultures (each strain individually) were inoculated in 10 ml of

TSBYE and incubated at 35°C for 24 hours. Strains were streaked on PALCAM (Difco) and tryptic soy agar with yeast extract (TSAYE) agar plates and incubated at 35°C for 24 hours to obtain isolated colonies (Bacteriological Analytical Manual, 1995).

Culture confirmation

Motility test: Motility test medium (Difco) and 10ml sterile Brain Heart Infusion (BHI) broth (Difco laboratories, Detroit, MI) were inoculated with the strains from TSBYE and incubated at 20-25°C. After incubation (16-18h), examination of a wet-mount preparation of BHI culture showed tumbling motility using the 100X objective of a phase-contrast microscope. Growth on a solid motility test medium, showed also an umbrella like shape of the microorganism (Bacteriological Analytical Manual, 1995).

Catalase test: Drops of the catalase reagent (3% H_2O_2) were added on TSAYE plate containing *L. monocytogenes* colonies and the formation of gas bubbles indicated a positive result for catalase test (Bacteriological Analytical Manual, 1995).

Sugar fermentation test: Each tube of sugar media containing rhamnose, xylose, and mannitol broth was inoculated with each strain and incubated at 35°C for 1-7 days. After 1, 2, 3, and 7 days, changes of color in the media (from purple to yellow) indicating fermentation of the carbohydrates by the organism (Difco Manual, 1998) confirmed that our strains were those of *L. monocytogenes* (Bacteriological Analytical Manual, 1995).

Gram reaction: A loopful of the BHI culture for each strain of *L. monocytogenes* was

smeared onto a slide and Gram test was performed. The results showed purple cells under optical microscope, indicated the retension of safranine dye in the membrane of the organism. This retension of dye, confirmed that the organism was a gram positive (Bacteriological Analytical Manual, 1995).

Christie-Atkins-Munch-Peterson (CAMP) test: For a CAMP test from the American Type Culture Collection (ATCC, Rockville, MD), *Staphylococcus aureus* (FDA strain ATTC 100) and *Rhodococcus equi* were streaked separately and vertically on sheep blood agar. The testing strain was then streaked horizontally between the two vertical strains on the agar without quite touching them. After 24-48 hours of incubation at 35°C, the plates were examined for hemolysis in the zone of influence of the vertical streaks. *L. monocytogenes* was enhanced near the *S. aureus* streak. This test was performed for each strain of *L. monocytogenes* (Bacteriological Analytical Manual, 1995).

Pork frankfurter preparation

The pork meat (30% fat) (Table III.1) used in this research was obtained from commercial trimmings and boneless picnics (Swift & Company, Omaha, NE). The frankfurters were prepared according to the formulation used in our Meat Science Laboratory (Table III.1). Sodium chloride, dry mustard, corn syrup solids, dextrose, sodium nitrite, sodium erythorbate, paprika, onion powder, garlic powder, coriander, and white pepper from AC Legg (Burningham, AL), and polyphosphate (sodium tripolyphospate and sodium hexametaphosphate) from Heller, Inc. (Bedford Park, IL) were common ingredients in the formulation of the frankfurters. Individual treatments

were also formulated to contain sodium acetate (0.25 or 0.50%, Sigma, St.Louis, MO), sodium lactate (3 or 6%, Purac Inc. 60% w/w, Lincolnshire, IL), sodium diacetate (0.25 or 0.50%, Niacet, Niagara Falls, NY), nisin (0.15%, Sigma, St. Louis, MO), glucono-delta-lactone (0.25%, Sigma, St. Louis, MO), monolaurin, (0.01%, Sigma, St. Louis, MO), lysozyme (0.01%, Sigma, St. Louis, MO), or allyl isothiocyanate (0.02%, Haarmann, & Reimer GmbH, Holzminden, Germany) (Tables III.2 and III.3).

Table III.1. List of ingredients used in the formulation of the frankfurters

Ingredient	Percent	Weight (g)
Pork Trim (30% fat)	82.19	9091.80
Water	10.00	1106.02
Dextose	2.00	221.20
Corn Syrup Solids	2.00	221.20
NaCl	2.00	221.20
Dry Mustard	0.90	99.52
Phosphate	0.40	44.22
Sodium Nitrite	0.02	1.72
Sodium Erythorbate	0.05	5.53
Paprika	0.25	27.65
Onion Powder	0.05	5.53
Garlic Powder	0.05	5.53
Coriander	0.05	5.53
White Pepper	0.05	5.53
TOTAL	100.00	11062.20

The meat and the spices, combined with each antimicrobial, as needed, were emulsified in a Meissner 35-L bowl chopper (RMF, Kansas City, MO) for 3-5 min (3000rpm blade speed, 10 rpm bowl speed) to a final temperature of 60°F (15.5°C). The meat batter was stuffed (Zesco Inc. Eden Prairie, MN) in 24 mm cellulose casings (Koch, Kansas City, MO), weighed for cooking yield (CY) and cooked in a smokehouse (Alkar, Lodi, WI) for

approximately 3 hours to an internal temperature of 155°F (70°C). The smokehouse cooking process consisted of 30 min dry air cycle of 175°F (80°C); 30 min liquid smoke

Table III.2. Percentage of antimicrobials in the formulation of frankfurters (Experiment 1)

Antimicrobials	Percent
Uninoculated control	0.00
Inoculated control	0.00
Sodium acetate	0.25
Sodium acetate	0.50
Sodium diacetate	0.25
Sodium diacetate	0.50
Sodium lactate	3.00
Sodium lactate	6.00

Table III. 3. Percentage of antimicrobials in the formulation of frankfurters (Experiment 2)

Antimicrobials	Percent
Uninoculated control	0.00
Inoculated control	0.00
Nisaplin (1500 IU)	0.15
Gluconodeltalactone	0.25
Lysozyme (100 ppm)	0.01
Allyl isothiocyanate (200ppm)	0.02
Monolaurin (100 ppm)	0.01

cycle; 30 min (140°F, 60°C) at a relative humidity of 26%; and 175°C (80°C) for the rest of the cooking time until the internal temperature of the frankfurters reached 155°F (70°C). A 5-min cold shower with tap water finished the process. The frankfurters were withdrawn from the smokehouse and stored overnight at 4°C in a cooler. After 12-16 hours of chilling, the frankfurters were weighed for cooking yield determination (CY $= \frac{G}{H} \times 100$, G, H, and CY represented the weight of the meat before cooking, the weight after cooking, and the cooking yield, respectively) and peeled manually. In order to simulate commercial frankfurters, the sticks of frankfurters were cut in 11 cm length

links, wrapped in wrapping paper and taken to the microbiology laboratory for inoculation with *L. monocytogenes.*

Product inoculation

After new cell suspensions of each strain were made, 1 ml of each strain was transferred and mixed together in a sterile test tube. Then, 1 ml of the culture mixture was added to 9 ml of sterile Phosphate Buffered Saline (PBS, Microbiology Laboratory Guide, 1998). Serial dilutions of this inoculum in sterile PBS tubes were made and plated (30°C, 24 hours) to determine the initial concentration of the inoculum. Two frankfurters from each treatment (n=24 bags containing 2 frankfurters each) were put in vacuum bags (20 x 25cm, 3mil STD barrier Nylon/polyethylene, Koch, Kansas City, MO) and brought under the hood for inoculation. Inoculum (0.2 ml), estimated to yield 10^3-10^4 cfu/cm^2, was dispensed on the surface of the two frankfurters in the bag. Using both hands, the frankfurters were rubbed from the outside of the bag in order to spread the inoculum throughout the surface of the frankfurters. Two controls (frankfurters with no antimicrobials) were used in this study; one was inoculated and the other not inoculated.

Product analysis

The inoculated and the control samples were stored at 4°C for 0, 10, 20, 35, 50, 70, 90, and 120 days. On each storage day (including time 0), samples (3 bags from each treatment) were pooled out from the cooler (4°C) and were transferred into corresponding stomacher bags and 100 ml of 0.1% peptone water were added to the bags and placed in a stomacher (Masticator, IUL Instruments, Barcelona, Spain) for 30 sec at 8.0 strokes per

sec for 30 sec. The samples were then diluted serially and plated in TSAYE and PALCAM agar (Difco) plates. The plates were incubated at 30°C for 48 hours and colonies were counted. The pH of the samples was taken by dipping the electrode (Denver Instrument Company, Arvada, CO) of the pH-meter (Denver Instrument Company, Arvada, CO) in the samples' bags after standardization of the pH-meter until the pH reader stabilized. After taking the pH, the bags were sterilized in an autoclave (121°C for 1 hour) and discarded.

Fat and moisture contents, and water activity of the frankfurters

Fat and moisture were determined following the AOAC International official methods 960.39 and 985.14, respectively, (AOAC, 1998). Water activity of slices of frankfurters from each treatment, was determined according to AOAC International official methods 978.18 (AOAC, 1998) with a water activity apparatus (Rotronic Instrument Corp, Huntington, NY). The formulas used for the fat and moisture contents, and the water activity were:

$$E = \frac{(A-B)*100}{A}, \quad F = \frac{[(B+C+0.0324)-D]*100}{A}, \text{ and} \quad X = \frac{Y-0.0862}{0.8896}$$

where

A= Weight of sample before drying

B= Weight of sample after drying

C= Weight of filter paper with one staple

D=Weight of sample after ether extraction

E= % moisture

F=% fat

0.0324= Weight of one staple

X= Expected value of the water activity

Y= measured value of the water activity

Statistical analysis

The first experiment, with frankfurters containing sodium acetate (0.25%), sodium diacetate (0.25%), and sodium lactate (3 or 6%) in the formulation was repeated three times. The second experiment including nisin (0.15%), lysozyme (0.01%), glucono-delta-lactone (0.25%), monolaurin (0.01%), and allyl isothiocyanate (0.02%) was not repeated. Three samples were analyzed per treatment and time in each replicate. Data were analyzed statistically with the SAS program (SAS, 1999) where analysis of variance and mean separation by the least squares means (General Linear Model procedure) were used. All analyses were done at 95% confidence level using treatment, time, media, replicate, and sample as class variables in the experimental model.

RESULTS AND DISCUSSION

Listeria monocytogenes counts (Figure III.1) increased more than 1.5 logs from 0 to 10 days in the inoculated control samples and reached over 10^8 cfu/cm^2 by day 50. With 6% sodium lactate added, growth of the pathogen was inhibited and the number of cfu/cm^2 decreased significantly ($p<0.05$) by 1.4 logs (Figure III.1, Table Appendix III 1) from day 0 to day 120. Likewise, 0.50% sodium diacetate inhibited and decreased the *L. monocytogenes* counts by 1.8 logs during storage at 4°C (Figure III.1, Table Appendix III 1). On the other hand, 3% sodium lactate inhibited growth of *L. monocytogenes* for at

least 50 days. Growth of the pathogen increased by 0.9 logs after 50 days compared to the inoculation level. Sodium diacetate at 0.25% and sodium acetate at 0.25 and 0.50% reduced growth of *L. monocytogenes* by 1 to 2 logs less than the control during storage (Figure III.1, Table Appendix III 1). On TSAYE, counts were higher (Figure III.2, Table Appendix III 2) for all treatments since the medium was not selective. The pH of the products did not change considerably except for frankfurters with sodium diacetate at 0.25 and 0.50% where it was reduced initially to 6.03 and 5.87 (Figure III.3, Table Appendix III 3), respectively, from the control pH value of 6.31. The pH of these products increased slightly during storage while the controls decreased and reached a value of 5.42 (Figure III.3, Table Appendix III 3).

Although the mechanism of the antimicrobial activity of lactates is not well understood, the inhibition of *L. monocytogenes* on the surface of frankfurters in the presence of sodium lactate (6 or 3%) may be explained by cytoplasmic acidification, specific ionic effect, water activity-lowering and chelating action (Ryser and Marth, 1999). Despite the optimal water activity (0.97) for growth of *L. monocytogenes*, the organism is able to multiply at a water activity as low as 0.90 (Ryser and Marth, 1999).

However, survival of the pathogen in processed foods may depend on the medium and its water activity, and the antibacterial chemicals in the formulation (Ryser and Marth, 1999). In addition, Johnson et al. (1988) predicted that growth in sausage-type products such as hard salami would be unlikely because of the bactericidal effect of the combination of salt, sodium nitrite, low pH in the product and the temperature of storage. Similar results were reported by Shelef (1994) who found that 4% sodium lactate was inhibitory to *L. monocytogenes* Scott A in comminuted beef stored at 20°C for 7 days.

Accordingly, Shelef and Yang (1991) indicated that potassium or sodium lactate (4%), decreased growth of *L. monocytogenes* in beef meat stored at 5°C for one week.

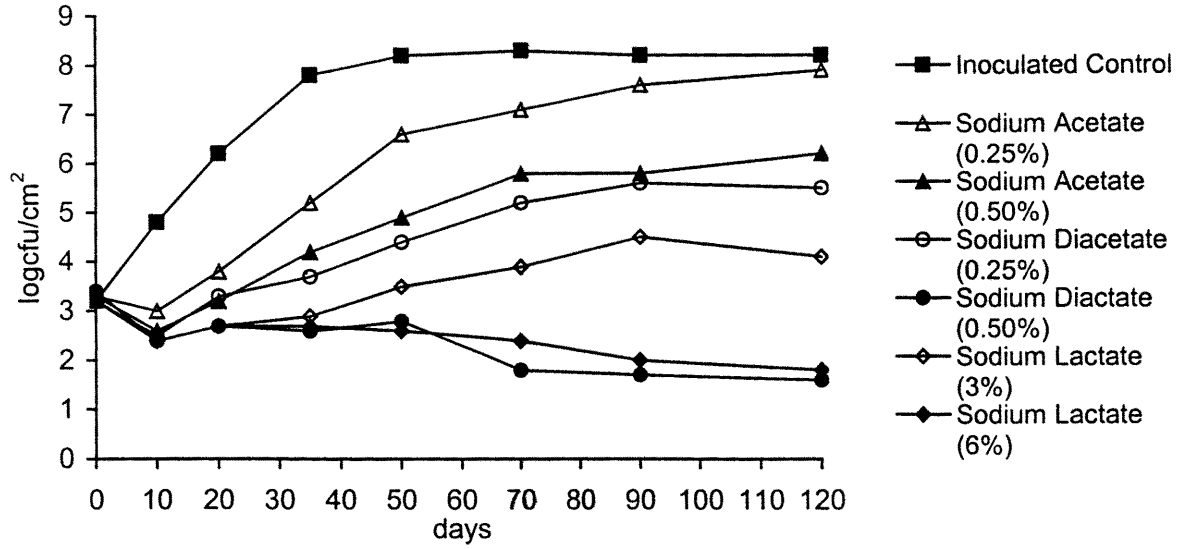

Figure III.1 (for Table Appendix III 1.). Changes (least squares means of log cfu/cm2, n=9) in populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C for 120 days (Standard Deviations varied from 0.0 to 2.9).

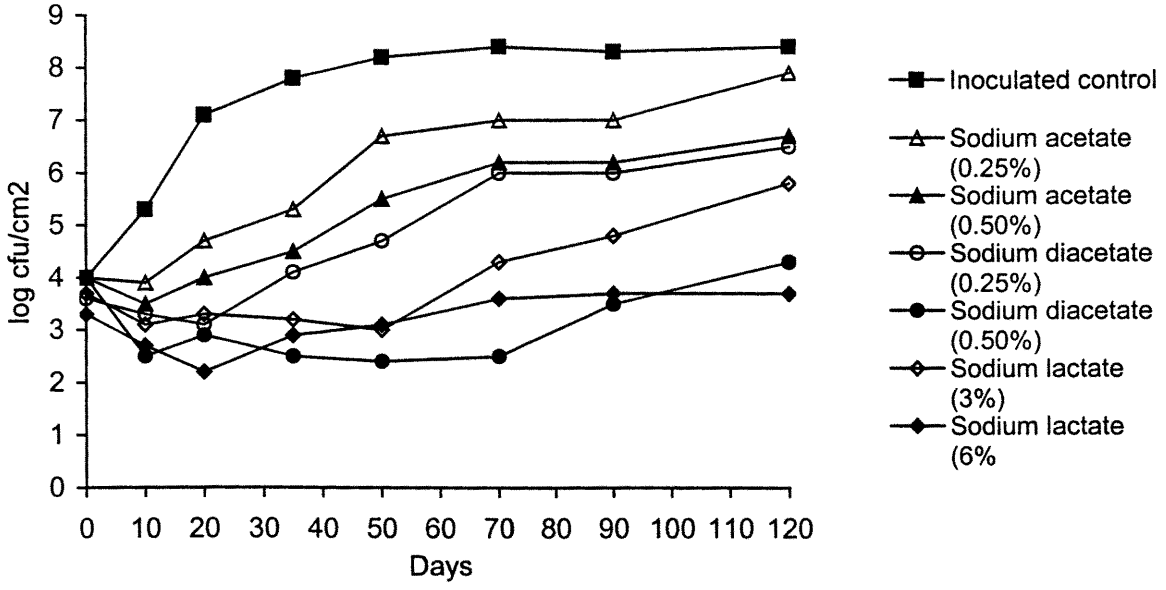

Figure III.2 (for Table Appendix III 2.). Changes (least squares means of log cfu/cm2, n=9) in bacterial counts (TSAYE agar) on the surface of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C for 120 days (Standard Deviations varied from 0.2 to 2.8).

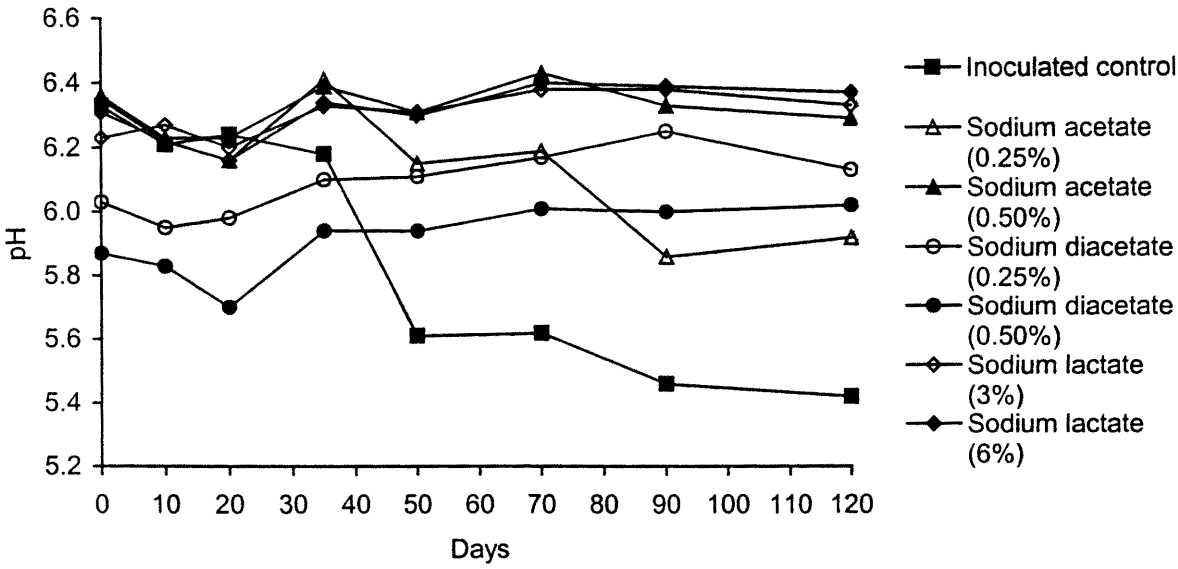

Figure III.3 (for Table Appendix III 3.). Changes in pH (least squares means; n=9) of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C for 120 days (Standard Deviations varied from 0.02 to 0.44).

When liver sausage containing 55% moisture and 2% NaCl was inoculated with *L. monocytogenes* and heat treated in a tube or heat processed in a water bath, and incubated at 5°C, inhibition of the organism was observed for 50 days when sodium, potassium, and calcium lactate (4%) were added to the liver sausage mixture (Weaver and Shelef, 1993). However, inhibition was enhanced in liver sausage with direct heat treatment, and calcium lactate (4%) inhibited *L. monocytogenes* more than sodium or potassium lactate (4%) according to the same authors.

When sodium diacetate (0.50%) is added to the formulation of the frankfurters, inhibition of *L. monocytogenes* may be attributed to its acidulant or antimicrobial effect. In fact, sodium diacetate is considered as generally recognized as safe (GRAS) by the FDA and is used as an antimicrobial agent in food (CFR, 1991; Shelef and Addala, 1994). In accordance with the results in this study, Shelef and Addala (1994) found that inhibition

of *L. monocytogenes* in BHI broth increased with increasing the diacetate levels (from 0 to 35 mmol/l) and decreasing incubation temperature (35, 20, and 5°C). As shown in Figure III 1(Table Appendix III 1), at 0.50%, sodium diacetate inhibited growth of the pathogen for 120 days while at 0.25% it did not. These results indicate that sodium lactate or sodium diacetate may inhibit growth of *L. monocytogenes* in cooked meat products but this inhibition may depend on the right concentration of the chemical, the composition of the product, and the storage temperature. When sodium lactate (3%) was added in the formulation (Table III.4), the water activity decreased by about 0.026 units from the control samples (0.972). As the percent of sodium lactate increased to 6%, the water activity decreased to 0.933. Sodium acetate (0.25 and 0.50%) and sodium diacetate (0.25 and 0.50%) decreased the water acivity, but there was no significant difference between the two antimicrobials regarding their effect on water activity ($P>0.05$). Decrease of the water activity was expected in this experiment since the chemicals added in the formulation of the frankfurters were salts, and acted as humectants. In addition, sodium lactate is well known as water activity-lowering agent (Ryser and Marth, 1999). While sodium lactate decreased the water activity of the product, none of the chemicals affected the moisture and fat contents, or the cooking yield (Table III.4). However, pH of product containing sodium diacetate (0.25 or 0.50%) was lower than that of other antimicrobials (Figure III.3, Table Appendix III 3). The other chemicals (nisin at 0.15%, allyl isothiocyanate at 0.02%, lysozyme at 0.01%, monolaurin at 0.01%, and glucono-delta-lactone at 0.25%) were not able to inhibit growth of *L. monocytogenes* during storage except for nisin, which held the growth for 10 days at 4°C (Figure III.4, Table Appendix III 4). Growth of the organism was higher on TSAYE agar (Figure III.5, Table

Appendix III 5) than on PALCAM agar and the pH was not affected (Figure III.6, Table Appendix III 6) by these antimicrobials. Moreover, none of the water activity, the moisture and fat content, or the cooking yield was affected by nisin, lysozyme, monolaurin, glucono-delta-lactone or ally isithiocyanate (Table III.5). As a matter of fact, growth of the pathogen reached approximately 10^8 cfu/cm^2 in 50 days. Nisin, a bacteriocin produced by certain strains of *Lactococcus lactis* subsp. *lactis*, has been proven to prevent growth of bacteria such as *Clostridium botulinum* in fermented dairy and meat products (Ryser and Marth, 1999). Research data indicated that, in order to inhibit growth of *L. monocytogenes*, minimum nisin concentrations of 1365, 2560, and 2496 ppm were required in TSAYE when the pH was adjusted to 5.5, 6.0, and 6.5, respectively (Ryser and Marth, 1999). Even though the concentration of nisin (1500 ppm) used in this study was below those mentioned above, it inhibited *L. monocytogenes* by 0.3 log for 10 days. According to Harris et al. (1989), resistance of *L. monocytogenes* to nisin may come from the ability of the organism to bind nisin rather than a specific gene coding for nisin resistance in plasmid DNA.

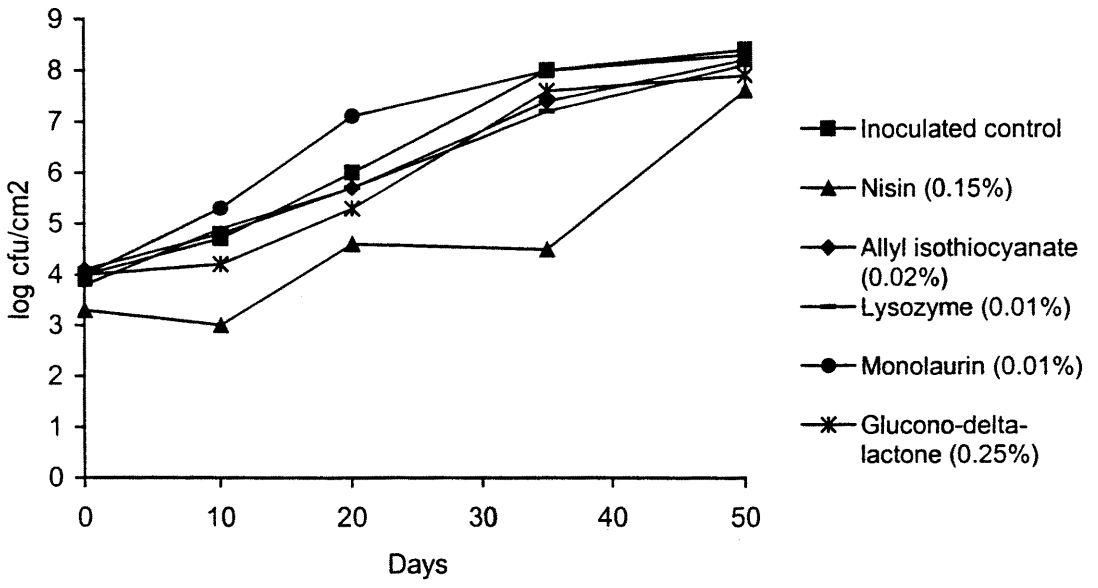

Figure III.4 (Table Appendix III 4.). Changes (least squares means of log cfu/cm^2, n=3) in populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C for 50 days (Standard Deviations varied from 0.0 to 0.7).

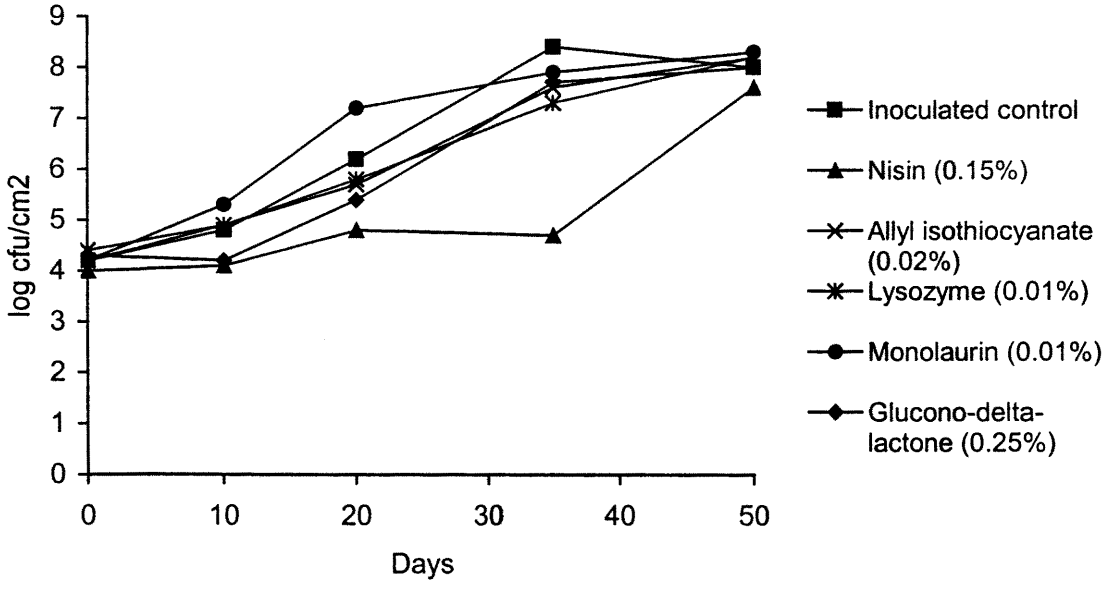

Figure III.5 (Table Appendix III 5.). Changes (least squares means of log cfu/cm^2, n=3) in total bacterial counts (TSAYE agar) on the surface of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C for 50 days (Standard Deviations varied from 0.0 to 0.7).

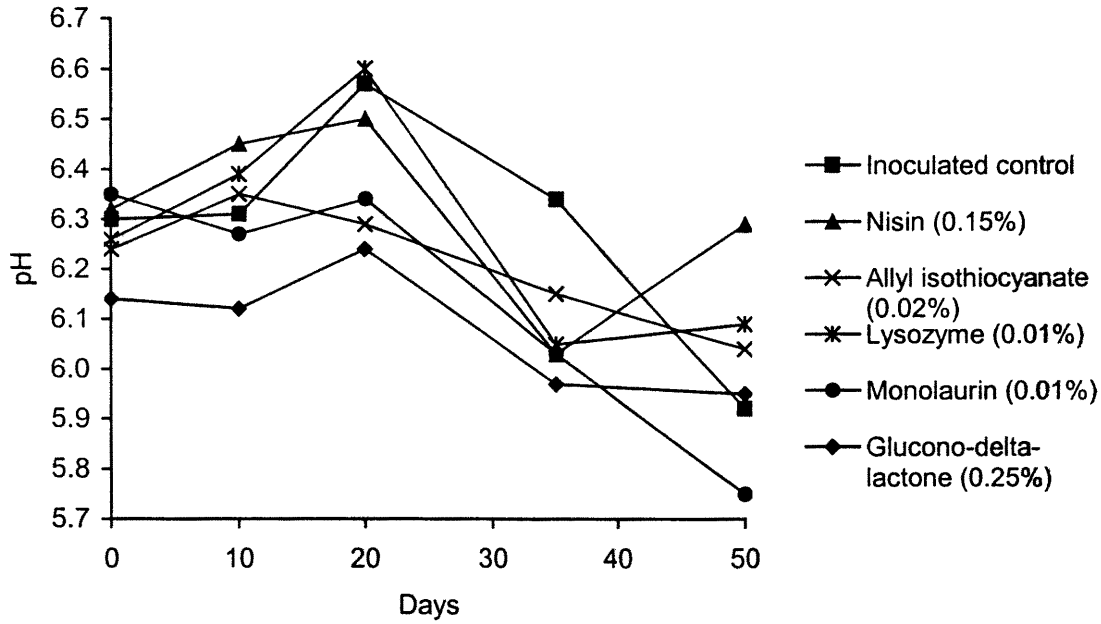

Figure III.6 (Table Appendix III 6.). Changes in pH (least squares means; n=3) of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C for 50 days (Standard Deviations varied from 0.00 to 0.51).

Table III.4. Means (Standard deviations) of cooking yield, water activity (a_w), moisture and fat contents (n=6) of frankfurters with antimicrobials in the formulation

Treatments	Cooking yield (%)	Water activity (a_w)	Moisture (%)	Fat (%)
Control	92.25	0.972 w	52.59	24.96
	(1.29)	(0.007)	(2.79)	(3.21)
Sodium acetate (0.25%)	91.55	0.964 wx	53.14	24.49
	(2.43)	(0.004)	(3.29)	(3.39)
Sodium acetate (0.50%)	89.89	0.969 wx	52.74	24.97
	(0.40)	(0.014)	(3.19)	(3.64)
Sodium diacetate (0.25%)	92.67	0.963 x	54.45	23.42
	(1.68)	(0.005)	(3.40)	(3.26)
Sodium diacetate (0.50%)	89.16	0.962 x	52.70	25.63
	(0.37)	(0.003)	(2.31)	(2.32)
Sodium lactate (3%)	90.83	0.946 y	53.06	22.97
	(0.42)	(0.006)	(1.85)	(2.57)
Sodium lactate (6%)	90.55	0.933 z	51.36	23.38
	(4.43)	(0.006)	(1.80)	(2.87)

wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
Means for moisture (%) and fat (%) and cooking yield (%) were not significantly different ($p>0.05$).

Table III.5. Means (Standard deviations) of cooking yield water activity (a_w), moisture and fat contents (n=3) of frankfurters with antimicrobials in the formulation

Treatments	Cooking yield (%)	Water activity (a_w)	Moisture (%)	Fat (%)
Control	85.65	0.965	54.59	22.04
		(0.002)	(1.14)	(1.00)
Nisin (0.15%)	85.97	0.968	54.91	22.43
		(0.002)	(0.06)	(0.48)
Allyl isothiocyanate (0.02%)	84.41	0.971	54.43	22.46
		(0.015)	(0.43)	(0.54)
Lysozyme (0.01%)	84.09	0.977	55.04	21.93
		(0.026)	(0.52)	(0.41)
Monolaurin (0.01%)	87.58	0.974	52.82	24.57
		(0.016)	(2.63)	(3.42)
Glucono-delta-lactone (0.25%)	77.41	0.965	55.30	21.58
		(0.004)	(0.08)	(0.09)

Means were not significantly different ($p>0.05$).
One sample for each treatment was used for cooking yield and the experiment was done only once, resulting in values without standard deviations.

CONCLUSIONS

The results indicated that when added to the formulation of frankfurters, sodium lactate at 6% and sodium diacetate at 0.50% inhibited growth of *Listeria monocytogenes* inoculated on the surface of product after peeling and before vacuum packaging for 120 days at 4°C. Sodium lactate at 3% inhibited growth of the pathogen for at least 50 days while sodium acetate at 0.25% or 0.50% and sodium diacetate at 0.25% inhibited growth for 20, 35, and 35 days, respectively. At 0.15%, nisin was inhibitory against *L. monocytogenes* for only 10 days, but did reduce growth during subsequent storage compared to the control. As applied in this study, lysozyme (0.01%), monolaurin (0.01%), glucono-delta-lactone (0.25%), and allyl isothiocyanate (0.02%) permitted growth of the pathogen during storage. Decrease in pH was observed in frankfurters containing sodium diacetate (0.25 or 0.50%) initially, as well as in the inoculated control during storage. The other

treatments did not affect the pH initially or during storage. The water activity of the products decreased as the concentration of sodium lactate increased from 3 to 6%, but the cooking yield, the moisture and fat contents were not affected by the antimicrobials. These results indicate that permissible levels of sodium diacetate and sodium lactate incorporated in the formulation of frankfurters inhibited growth of *L. monocytogenes* for 35 and 50 days, respectively, at 4°C while accepted level of sodium acetate inhibited the pathogen for 20 days only at the same temperature. By doubling the permissible concentrations of these additives, growth of the organism was completely inhibited with sodium diacetate and sodium lactate. Increase of the permissible levels of additives used in this study may be avoided by combining the permissible concentrations of antimicrobials in the formulation of frankfurters. This hypothesis was tested in the studies reported in Chapter IV.

CHAPTER IV

EFFECT OF COMBINATION OF ANTIMICOBIALS IN THE FORMULATION AND POST-PACKAGING THERMAL PASTEURIZATION ON *LISTERIA MONOCYTOGENES* IN FRANKFURTERS VACUUM PACKAGED AND STORED AT 4°C

ABSTRACT

Contamination of ready-to-eat foods, such as frankfurters, with *Listeria monocytogenes* is a major concern that needs to be addressed in order to enhance the safety of these products. The objective of this study was to determine the effective combinations of antimicrobials incorporated in the formulation, in previous experiment, against *L. monocytogenes* inoculated (10^3-10^4 cfu/cm^2) on their surface after peeling and before vacuum packaging. In addition, the antimicrobial effect of dipping the packaged products, in hot (75-80°C) water (30-90 sec) was evaluated. Samples were stored at 4°C for up to 50 or 120 days and periodically analyzed for microbial growth on trypsic soy agar plus 0.6% yeast extract (TSAYE) and PALCAM agar. Products were also tested for pH, water activity, moisture and fat contents. While sodium lactate at 3% inhibited

growth of *L. monocytogenes* for 35-50 days, its combination with sodium acetate at 0.25%, sodium diacetate at 0.25% or glucono-delta-lactone at 0.25% inhibited or reduced bacterial growth (average population changes on PALCAM: –0.8 to –1.3 log cfu/cm^2) throughout storage. Dipping of packaged frankfurters in hot water enhanced the antilisterial effect of combined chemical additives on stored products by delivering 0.4-0.9 log cfu/cm^2 immediate reductions in *L. monocytogenes* counts at treatment. However, dipping frankfurters containing no additives in hot water did not inhibit pathogen growth for more than 10-20 days, unless one frankfurter was placed per bag and heat-treated for 90 sec with an inhitial reduction of 1.2 logs. The initial thermal reduction of *L. monocytogenes* in frankfurters without antimicrobials was higher with one frankfurter per bag than with two frankfurters per bag when dipped in hot water (75 or 80°C). Moreover, dipping the frankfurters in hot water for 60 or 90 sec resulted in a higher initial reduction of listerial counts than dipping for 30 sec. When dipped at 80°C, there was a similar reduction on one frankfurter per bag at 30, 60, and 90 sec. However, dipping for 90 sec was more efficient immediately than 60 and 30 sec on two frankfurters per bag. The pH of frankfurters with no additives decreased dramatically after 35 days in all treatments during storage whereas with combined antimicrobials in the formulation there was an immediate decrease of the pH, which was maintained during storage, in formulations of sodium lactate (3%) combined with sodium diacetate (0.25%) or glucono-delta-lactone (0.25%). The cooking yield and the water activity were not affected by the combinations of antimicrobials while sodium lactate (3%) combined with sodium diacetate (0.5%) decreased the moisture content by 3 to 4% and increased the fat content by the same amount. These results indicate that inclusion of 3% sodium lactate combined with 0.25%

sodium acetate, sodium diacetate, or GDL in a cured meat formulation may control *L. monocytogenes* growth during refrigerated (4°C) storage. This protective effect may be enhanced by post-packaging thermal treatment.

INTRODUCTION

Several compounds used in cured meat products have an antimicrobial effect on the common flora of the product. Salt (NaCl), nitrite, and spices are common ingredients used in the formulation of products such as frankfurters. However, *Listeria monocytogenes* is able to grow under stressful conditions such as in a broth supplemented with 10% NaCl (Ryser and Marth, 1999). The pathogen, however, has been found in meat products such as sausages, or hot dogs that contain salt, spices and other compounds that have an antimicrobial effect against a large range of bacteria. Thus, those compounds are not necessarily bacteriostatic or bactericidal to pathogens such as *L. monocytogenes*. Combinations of approved additives with antimicrobials such as sodium diacetate, sodium acetate, and sodium lactate at higher concentrations in the formulation may provide a lethal effect on *L. monocytogenes* in vacuum packaged frankfurters stored at refrigerated temperatures.

The objective of the study reported in this chapter was to evaluate growth, during storage at 4°C, of *L. monocytogenes* in frankfurters formulated with combinations of sodium lactate (3%) with sodium acetate (0.25%), sodium diacetate (0.25%), and glucono-delta-lactone (0.25%) and exposed to a post-packaging thermal pasteurization treatment. Avoiding increase of permissible levels of antimicrobials in the formulation, by combining permissible concentrations of antimicrobials used in he previous chapter, may

inhibit completely growth of *L. monocytogenes* on vacuum packaged frankfurters stored at 4°C.

MATERIALS AND METHODS

Pork Frankfurter preparation

All the ingredients used in the formulation of frankfurters were the same as those listed in Table III.1. The frankfurters were also processed, cooked, cut, and inoculated, as in the previous experiment (Chapter III).

Table IV.1.Post-packaging thermal treatment of frankfurters (dipped in hot water) at 75-80°C in vacuum packages.

Treatments
Inoculated, control, not dipped, 1 frank/bag
Inoculated, control, not dipped, 2 franks/bag
Inoculated, dipped 30 sec, 1 frank/bag
Inoculated, dipped 30 sec, 2 franks/bag
Inoculated, dipped 60 sec, 1 frank/bag
Inoculated, dipped 60 sec, 2 franks/bag
Inoculated, dipped 90 sec, 1 frank/bag
Inoculated, dipped 90 sec, 2 franks/bag

Frankfurters were inoculated with 0.1ml (one frankfurter per bag) or 0.2ml (two frankfurters per bag) of a composite of ten strains of *L. monocytogenes*. After inoculation, as described above in Chapter III, the frankfurters were vacuum packaged, heated by dipping in hot water (75-80°C) for 0-90 sec (Table IV.1), and stored at 4°C.

In the second phase of the study, antimicrobials were added in the formulation to produce six treatments of frankfurters (Table IV.2). The frankfurters were prepared like those with single antimicrobials of Chapter III. However, in this case, sodium lactate (3%) was

added alone or mixed with sodium acetate (0.25%), sodium diacetate (0.25%), or glucono-delta-lactone (0.25%), and after cooking and cutting, the frankfurters were placed in vacuum bags (two per bag) and inoculated with 0.2ml of the composite inoculum.

Table IV.2. Combinations of antimicrobials in the formulation of the frankfurters dipped or not dipped in hot water (80°C) for 60 sec after inoculation with *L. monocytogenes* and vacuum packaging.

Antimicrobial	Percent
Uninoculated control	0.00
Inoculated control	0.00
Sodium lactate	3.00
Sodium lactate + sodium acetate	3.00 + 0.25
Sodium lactate + sodium diacetate	3.00 + 0.25
Sodium lactate + glucono-delta-lactone	3.00 + 0.25

One group of inoculated frankfurters was dipped in hot water (176°F, 80°C) and another group was not dipped. The experiment was then conducted exactly like in Chapter III, including the determination of the water activity, the cooking yield, the moisture and fat contents of the products.

Product immersion in hot water

Two groups of samples (1 or 2 frankfurters per bag) were dipped in hot water in a water bath previously set at the proper temperature (75-80°C) after vacuum packaging. The temperature of the water was read with a thermometer placed in the water bath. Each sample, except for the control, was immersed in the hot water of the water bath for 30 to 90 seconds and removed immediately after. Samples were stored at 4°C for 0, 10, 20, 35, 50 days for the first replicate and 0, 10, 20, 35, 50, 70 days for the second replicate.

Bags of two frankfurters per bag with two antimicrobials in the formulation were dipped in hot water (176°F, 80°C) including the controls for this group. They were stored at 4°C for 0, 10, 20, 35, 50, 70, 90, and 120 days. The other bags containing two frankfurters were not dipped in hot water and were stored at 4°C after vacuum packaging. Analysis for day zero was performed immediately.

RESULTS AND DISCUSSION

Samples containing two or one frankfurter, without antimicrobials, and dipped in hot water (75°C) after vacuum packaging.

Initial reductions of 1.96, 3.02, and 3.07 log cfu/cm^2 were observed in *L. monocytogenes* counts when one frankfurter per bag was dipped in hot water (75°C) for 30, 60, and 90sec, respectively, while for the same dipping time, initial reductions with two frankfurters per bag were 0.90, 1.07, and 1.07 log cfu/cm^2, respectively (Table IV.3). Moreover, Dipping one frankfurter per bag in hot water (75°C) for 60 or 90sec reduced *L. monocytogenes* counts more significantly ($p<0.05$) than dipping them for 30sec (Table IV.3).

Higher initial reduction with one frankfurter per bag samples was probably due to the surrounded hot water which injured the cells on the surface of the product, while with two frankfurters per bag, the hot water could not reach the microorganism located at the inter-surface between two frankfurters, making it ineffective for inhibition.

When dipped in hot water at 80°C, initial reductions were still higher in one frankfurter per bag than in two frankfurters per bag but reductions were not significantly different

($p>0.05$) in the former (Table IV. 4). However, with two frankfurters per bag, initial reduction at 90sec was significantly different ($p<0.05$) than that at 60 or 90sec

Table IV.3. Means (Standard Deviations) of initial reductions (3.9 logs of cfu/cm^2 on control) of populations (n=3) of *L. monocytogenes* (PALCAM agar) on the surface of vacuum packaged frankfurters with no antimicrobials after dipping in hot water (75°C).

Dipping time (sec)	Reduction of *L. monocytogenes* counts by the thermal treatment	
	One frankfurter/bag	Two frankfurters/bag
30	1.96 zB	0.90 zA
	(0.38)	(0.07)
60	3.02 yC	1.07 zA
	(0.05)	(0.14)
90	3.07 yD	1.07 zA
	(0.06)	(0.36)

y z: means within a column lacking a common superscript letter differ ($p<0.05$).
A B C D: means within a row lacking a common superscript letter differ ($p<0.05$).

(Table IV. 4). The overall reduction of listerial counts in hot water was unexpectedly lower at 80°C than at 75°C. This unexpected effect of the hot water may be due to many reasons including the texture of the frankfurters, which may protect hidden pathogen cells, or to the fact that the number of bag dipped at a time was higher in hot water at 80°C than at 75°C, preventing the heat to penetrate deeply in the samples.

L. monocytogenes counts in inoculated controls not dipped (1/bag or 2/bag) in hot water (75°C) increased ($p<0.05$) and reached very high counts (>10^7 cfu/cm^2) after 35 days regardless of the number of frankfurters in each bag (Figures IV.1, Table Appendix IV 1). In addition, when dipped for 30, 60, 90sec, all samples (1/bag or 2/bag) showed an increase in *L. monocytogenes* counts (PALCAM agar), except when one frankfurter per bag was dipped in hot water for 90sec, where inhibition was exhibited during storage (50

days). Although there was growth in all samples, differences have been found between 1/bag and 2/bag samples (Figure IV.1, Table Appendix IV 1).

Table IV.4. Means (Standard Deviations) of initial reductions (4.0 logs of cfu/cm^2 on control) of populations (n=3) of *L. monocytogenes* (PALCAM agar) on the surface of vacuum packaged frankfurters with no antimicrobials after dipping in hot water (80°C).

Dipping time (sec)	Reduction of *L. monocytogenes* counts by the thermal treatment	
	One frankfurter/bag	Two frankfurters/bag
30	1.25 zCD	0.31 zA
	(0.21)	(0.16)
60	0.96 zBCD	0.55 zAB
	(0.37)	(0.13)
90	1.28 zD	0.86 yBC
	(0.31)	(0.15)

y z; means within a column lacking a common superscript letter differ ($p<0.05$).
A B C D: means within a row lacking a common superscript letter differ ($p<0.05$).

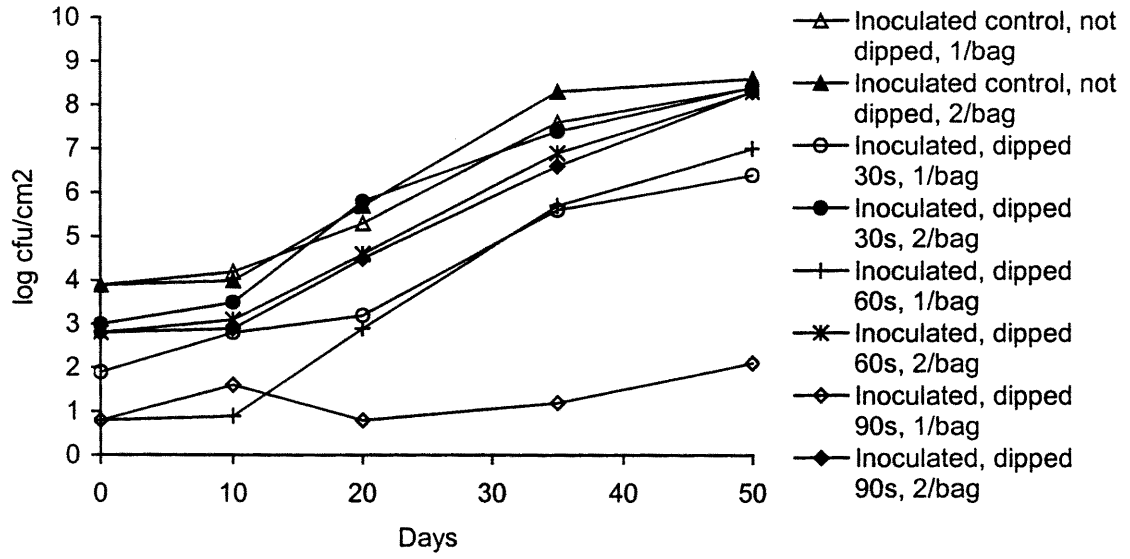

Figure IV.1 (Table Appendix IV 1). Changes (least squares means of log cfu /cm2, n=3) in means (Standard Deviation) of *L. monocytogenes* (PALCAM agar) on the surface of frankfurters without antimicrobials in the formulation, vacuum packaged, dipped in hot (75°C) water (30-90sec.) and stored at 4°C for 50 days (Standard Deviations varied from 0.0 to 1.5).

Bacterial growth on TSAYE agar was generally higher than growth on PALCAM agar, and one per bag frankfurters indicated lower growth than two per bag frankfurters (Figure IV.2, Table Appendix IV 2). The pH for all products started to decrease dramatically after day 35, as the growth of the microorganisms reached 10^7-10^8 cfu/cm^2 (Figure IV.3, Table Appendix IV 3).

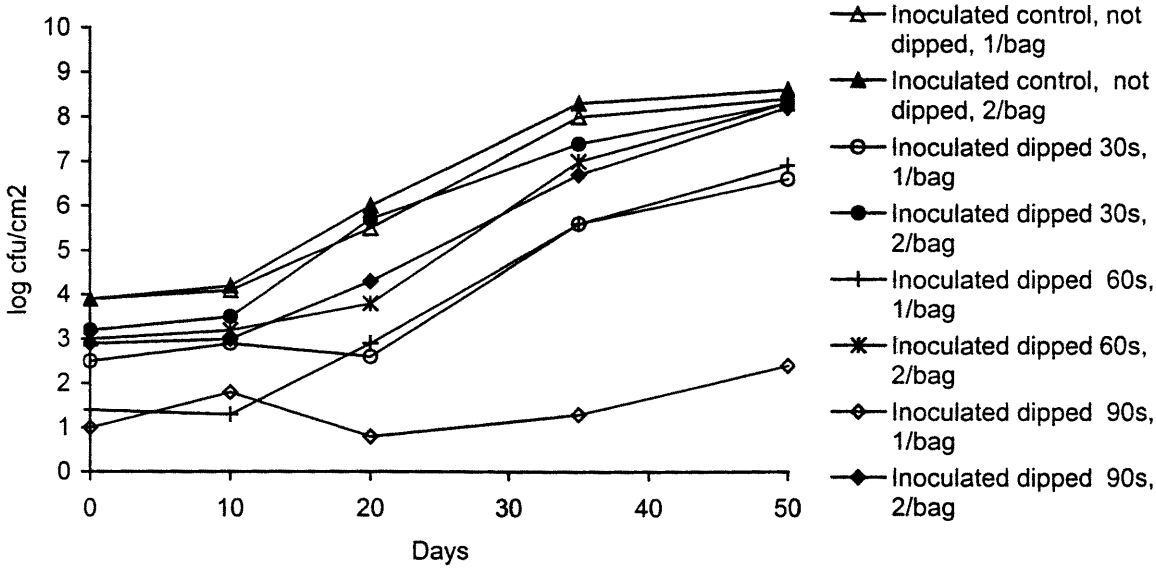

Figure IV.2 (Table Appendix IV 2). Changes (least squares means of log cfu/cm2, n=3) in means (Standard Deviations) of bacterial counts (TSAYE agar) on the surface of frankfurters without antimicrobials in the formulation, vacuum packaged, dipped in hot (75°C) water (30-90sec.) and stored at 4°C for 50 days (Standard Deviations varied from 0.1 to 2.3).

Listeria monocytogenes counts in inoculated controls not dipped (1/bag or 2/bag) in hot water (80°C) increased ($p<0.05$) and reached very high counts (>10^6 cfu/cm^2) after 35 days (Figures IV.4 and IV.5, Tables Appendix IV 4 and 5). When dipped in hot water (80°C) for 30, 60, 90 sec, all samples (1/bag or 2/bag) showed an increase in *L. monocytogenes* counts (PALCAM agar), except when one frankfurter per bag was dipped in hot water for 90 sec with inhibition for 20 days, after that growth increased and

decreased later during storage. This unexpected increase in *L. monocytogenes* counts (more than with one frankfurters per bag, dipped in hot water at 75°C) may be due to a lack of similarity in the dipping procedure, which was not performed by the same operator for both temperatures (80 and 75°C). Moreover, samples of frankfurters were not dipped individually in the water bath; the heat transfer from the hot water to the samples may not be the same between the bags at the periphery of the lot of samples and those in the center of the lot during the dipping process. Growth in one frankfurter per bag samples was also lower than that in two frankfurters per bag samples (Figure IV.4, Table Appendix IV 4). The pH (Figure IV.6, Table Appendix IV 6) increased during storage (70 days).

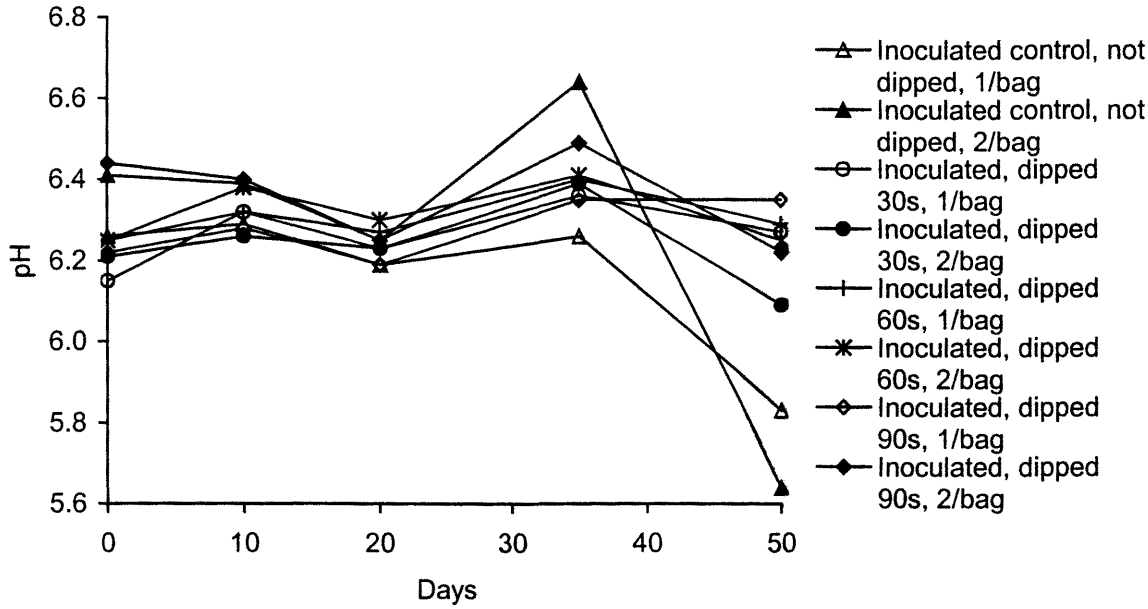

Figure IV.3 (.Table Appendix IV 3) Changes in pH (least squares means; n=3) of frankfurters without antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot (75°C) water (30-90sec.) and stored at 4°C for 50 days (Standard Deviations varied from 0.01 to 0.49).

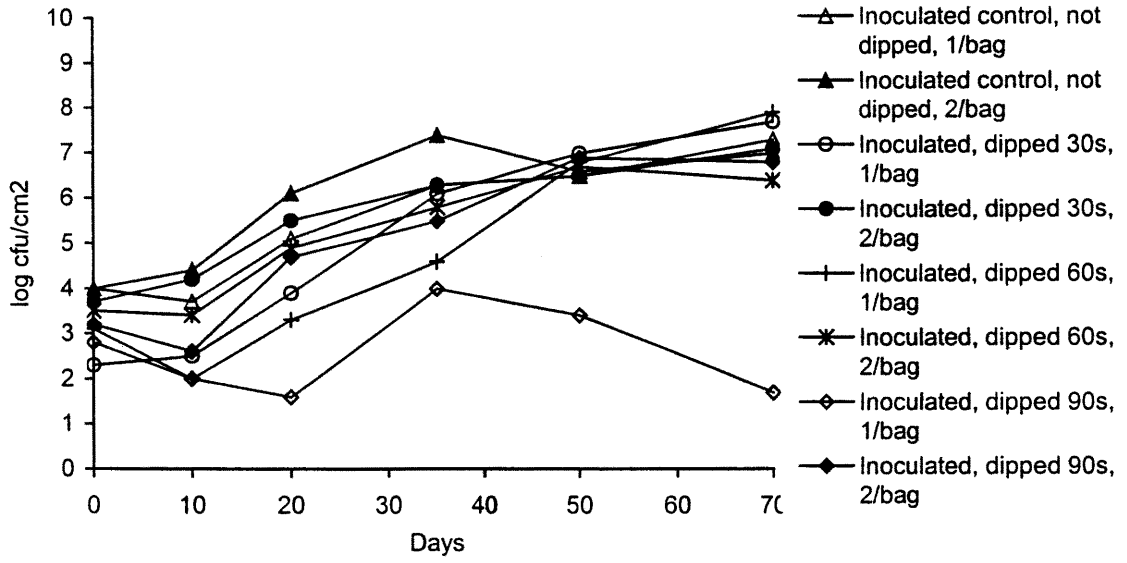

Figure IV.4 (Table Appendix IV 4). Changes (least squares means of log cfu/cm^2, n=3) in means (Standard Deviations) of *L. monocytogenes* (PALCAM agar) on the surface of frankfurters without antimicrobials in the formulation, vacuum packaged, dipped in hot (80°C) water (30-90sec.) and stored at 4°C for 70 days (Standard Deviations varied from 0.0 to 2.7).

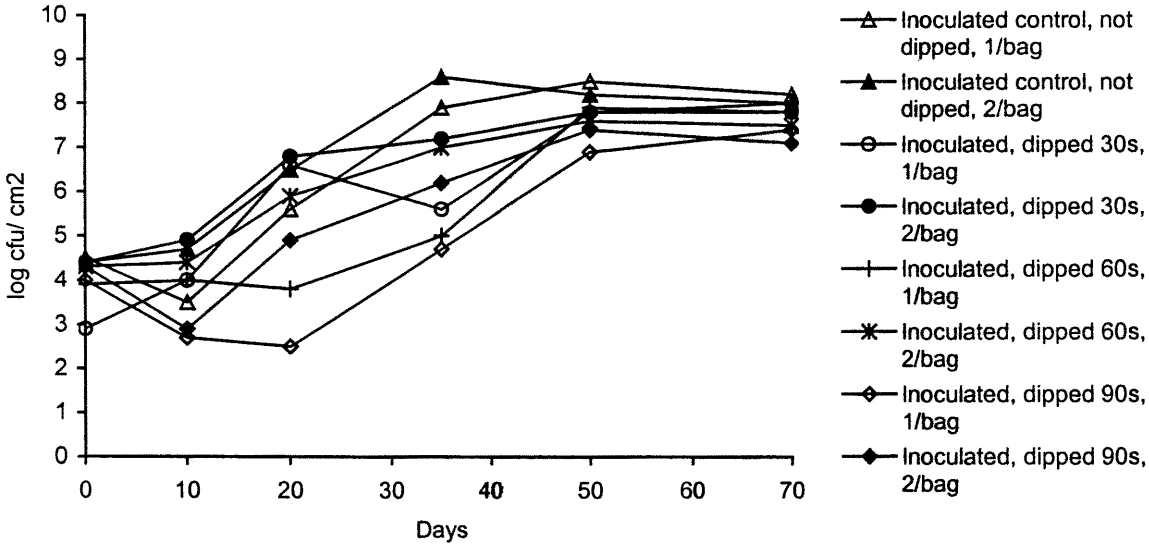

Figure IV.5 (Table Appendix IV 5). Changes (least squares means of log cfu/cm^2, n=3) in means (Standard Deviation) of bacterial counts (TSAYE agar) on the surface of frankfurters without antimicrobial in the formulation, vacuum packaged, dipped in hot (80°C) water (30-90sec.) and stored at 4°C for 70 days (Standard Deviations vary from 0.1 to 2.3).

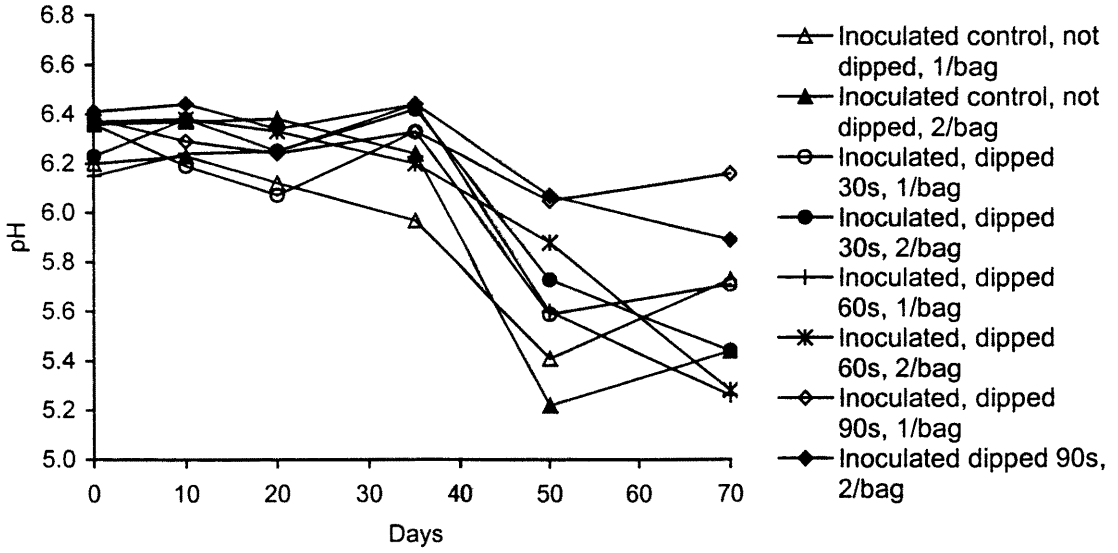

Figure IV.6 (Table Appendix IV 6). Changes in pH (least squares means; n=3) of frankfurters without antimicrobial in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot (80oC) water (30-90sec.) and stored at 4°C for 70 days (Standard Deviations vary from 0.01 to 0.63).

The water activity (0.962 ± 0.00), and the cooking yield (0.88.53 ± 1.09) of the frankfurters, and the moisture (52.52 ± 0.66) and fat (23.31 ± 1.31) contents of the meat were similar to those of the control (no additives) in the experiment with antimicrobials in the formulation (Chapter III).

Boyle et al. (1990), reported that heating of inoculated ground beef (80% lean), previously stored at 4°C for 48h to 50, 60 and 65°C resulted in 0.4-0.6, 2.1-2.3 and 4.6-5.5 logs reduction of *L. monocytogenes* from the inoculated control sample (7.84 logs cfu/g), respectively. Another study (Coote et al., 1991) showed that food should be maintained for 2 min at 70°C in order to observe a substantial reduction in the number of *L. monocytogenes*. Vacuum-packaged summer sausage surface inhoculated with *L. monocytogenes* (10^8 cfu/ml), was heated to determine the D-values at 66, 77, 88, and 99°C (Roering et al., 1998). The results indicated a reduction of about 3 logs cfu/g within

30, 60, or 90 sec at 99, 88, or 77°C, respectively. However, heating at 66°C for 240 sec reduced the counts of the pathogen by less than 2.0 log cfu/g. The authors found D-values of 2.08, 0.84, 0.37, and 0.28min at 66, 77, 88, and 99°C, respectively, indicating the effectiveness of pasteurization of vacuum-packaged summer sausage against *L. monocytogenes*. Glass and Doyle (1989) reported that processed meat products including ham, bologna, sliced chicken, siled turkey, fermented semidried sausage, bratwurst, and cooked roast beef, inoculated with *L. monocytogenes* (10^5 cfu/g), vacuum packaged, and stored at 4°C for 6 weeks, indicated inhibition of the pathogens in the fermented sausage but did not in the other meat products. According to the authors, the inhibition was due to the low pH in the fermented sausage (pH 4.78 to 5.06) during storage. Fermented beaker sausage inoculated (10^8-10^9cfu/ml) with a mixture of five strains of *L. monocytogenes*, vacuum-sealed, and heated in a water bath at 48.9, 51.7, 54.4, 52.2, and 60.0°C indicated D-values of 98.6, 44.4, 20.1, 11.2, and 9.13min, respectively (Schoeni et al., 1991). In the same study, ground beef inoculated with only one strain of *L. monocytogenes* and heated at 54.4, 57.2, 60.0, and 62.0°C resulted in D-values of 12.5, 3.41, 1.62, and 0.73min, respectively, four time less than with five strains.

Yen et al. (1992) found that a complete meat cure added to ground pork reduced the thermal destruction of *L. monocytogenes* at temperatures below 67°C. According to the same authors, sodium chloride 2-2.5% reduced thermal destruction of the pathogen on groud pork at temperatures of 50-60°C but not above 65°C. In a recent study on thermal inactivation of *L. monocytogenes* (Bolton et al., 2000), inoculated vauum-packaged minced beef (10g) and minced beef inoculated in a vacutainer (10g) were heated at 50, 55, and 60°C in a water bath. Vacuum-packaged minced beef and solid beef (50g) were

also heated in a commercial retort at 48, 52, and 56°C. The results indicated that D-values of vacuum-packaged minced beef (3.2 and 0.15 min) were lower than those of minced beef in vacutainer (3.4 and 0.31 min) at 55 and 60°C, respectively. In addition, when the products were cooked in a commercial retort at 48, 52, and 56°C, D-values of vacuum-packaged minced beef (74.1, 16.3, and 2.3 min) were also lower than those of solid beef (88.6, 26.7, and 3.1 min), respectively. The authors concluded that the differences in D-values may be due to variation between strains of *L. monocytogenes* or the heating rate in the retort may be slower in solid beef than in vacuum-packaged minced beef allowing in the latter a rapid destruction of the pathogen.

Samples containing frankfurtesr with combined antimicrobials in the formulation dipped or not dipped in hot water (80°C) after vacuum packaging.

There was a slight initial thermal reduction of *L. monocytogenes* counts due to the dipping treatment (hot water at 80°C) but there was no significant difference between treatments (Table IV.5). It appears from this result that the heating effect (80°C for 60 sec) did not affect significantly growth of the pathogen. However, when the heating treatment was associated with the combined antimicrobials, growth in control frankfurters dipped or not dipped in hot water reached 10^7 cfu/cm^2 after 35 days (Figure IV.7, Table Appendix IV 7). When combinations of sodium lactate (3%) with sodium acetate (0.25%), sodium diacetate (0.25%), or gucono-delta-lactone (0.25%) were added to the formulation of the frankfurters, inhibition or reduction of growth of *L. monocytogenes* was approximately 1log (PALCAM) during storage at 4°C (Figure IV.7 and IV.8, Tables Appendix IV 7 and 8). Sodium lactate singly, inhibited growth for 35-50

days in both cases when the frankfurters were not dipped and dipped (Figure IV.7 and IV.8, Tables Appendix IV 7 and 8). The combination of sodium lactate (3%) sodium acetate (0.25%), glucono-delta-lactone (0.25%), or sodium diacetate (0.25%) increased *L. monocytogenes* inhibition during storage. As showed earlier in Chapter III, sodium lactate and sodium diacetate are respectively a water activity-lowering agent and an acidulant, and may affect the pathogen's growth as combined hurdles. Glucono-delta-lactone is an acidic form of lactate also called D-gluconic acid lactone able to produce acid in the food environment at a lower level than sodium diacetate. When in addition to the two antimicrobials in the formulation, the frankfurters were dipped in hot (176°F, 80°C) water after vacuum packaging, products with sodium lactate (3%) combined with sodium diacetate (0.25%) or glucono-delta-lactone (0.25%) showed a total inhibition

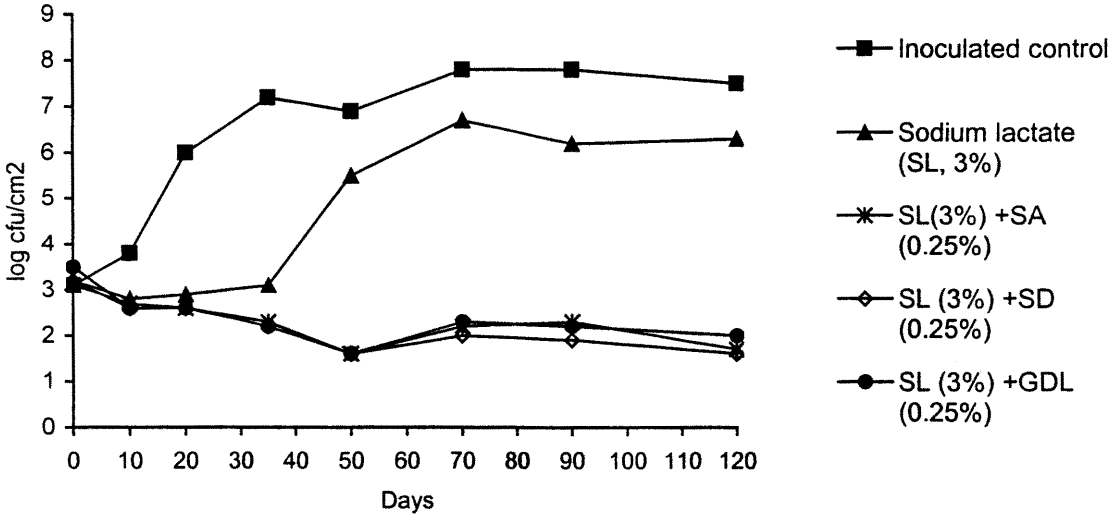

Figure IV.7 (Table Appendix IV 7). Changes (least squares means of log cfu/cm2, n=6) in mean populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with combined antimicrobials in the formulation, vacuum packaged, not dipped in hot water, and stored at 4°C for 120 days (Standard Deviations varied from 0.01 to 1.7). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

and a listericidal activity (PALCAM) with a reduction of 1.5 logs during storage

(Figure IV.8, Table Appendix IV 8). Likewise, products with combination of sodium lactate (3%) and sodium acetate (0.25%) indicated a bacteriostatic effect on *L. monocytogenes* when dipped in hot water. Although the dipping effect was effective by reduced the initial count by approximately 0.6 log, sodium lactate singly inhibited *L. monocytogenes* for about 35-50 days. In addition to their acidity and water activity lowering effect, the chemicals added in the formulation, the heat of water may act as an additional hurdle against the growth of *L. monocytogenes* on the surface of the frankfurters. There was no major difference in TSAYE counts on both dipped and not dipped products (Figure IV.9 and IV.10, Tables Appendix IV 9 and 108). The pH of the products was not affected considerably (pH 6.0) except for the inoculated control where the pH decreased to approximately 5.3 or 5.6 during storage (Figure IV.11 and IV.12, Tables Appendix IV 11 and 12).

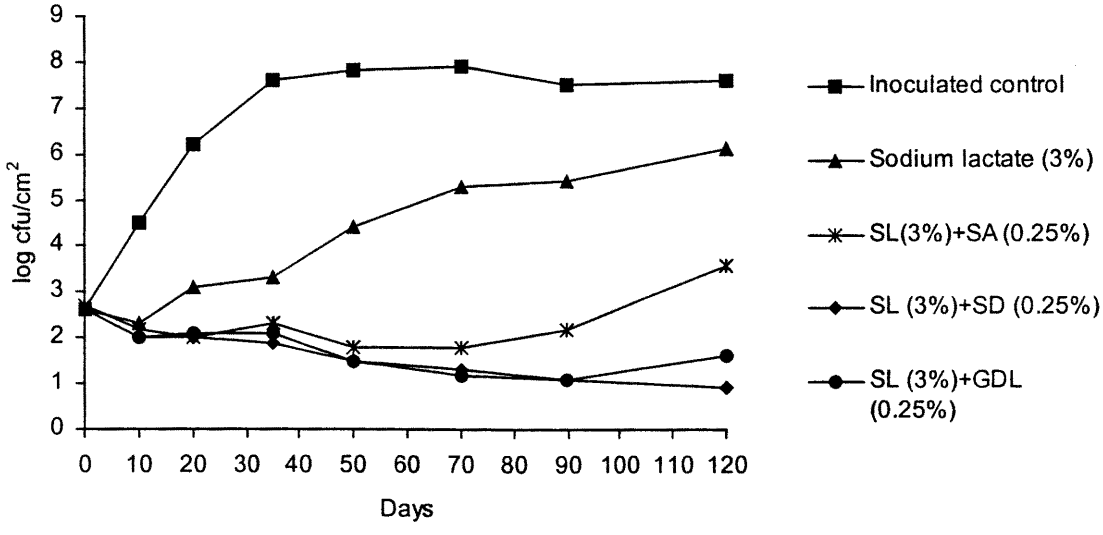

Figure IV.8 (Table Appendix IV 8). Changes (least squares means of log cfu/cm2, n=6) in mean populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with combined antimicrobials in the formulation, vacuum packaged, dipped in hot water (80°C) and stored at 4°C for 120 days (Standard Deviations vary from 0.1 to 2.2). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

However, products containing combinations of sodium lactate (3%) plus sodium diacetate (0.25%) or glocono-delta-lactone (0.25%) showed a lower pH compared to the other treatments (Figure IV.11 and IV.12, Tables Appendix IV 11 and 12). Combinations of antimicrobials in the formulation did not affect the cooking yield, the water activity, the moisture or the fat content during storage (Table IV.6). However, a higher fat content batch may be the source of the slight increase of the fat percent in product containing sodium lactate (3%) combined with sodium diacetate (0.50%). Reduction of *L. monocytogenes* counts was found in all treatments, but it was greater in frankfurters containing sodium lactate (3%) combined with glucono-delta-lactone (0.25%) or sodium diacetate (0.25%), after dipping in 80°C (Table IV.6). Combination of sodium acetate (0.25%) and sodium lactate (2.5%) in vacuum packaged sausage and cooked ham inoculated with *L. monocytogenes* resulted in inhibition of the pathogen for 4 to 6 weeks at 4°C (Blom et al., 1997).

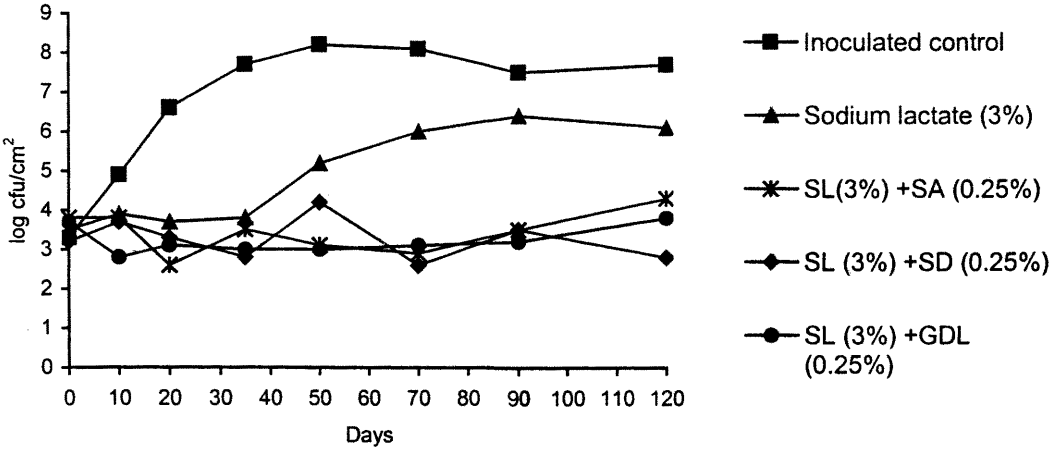

Figure IV.9 (Table Appendix IV 9). Changes (least squares means of log cfu/cm2,n=6) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, not dipped in hot water, and stored at 4°C for 120 days (Standard Deviations vary from 0.1 to 3.2). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

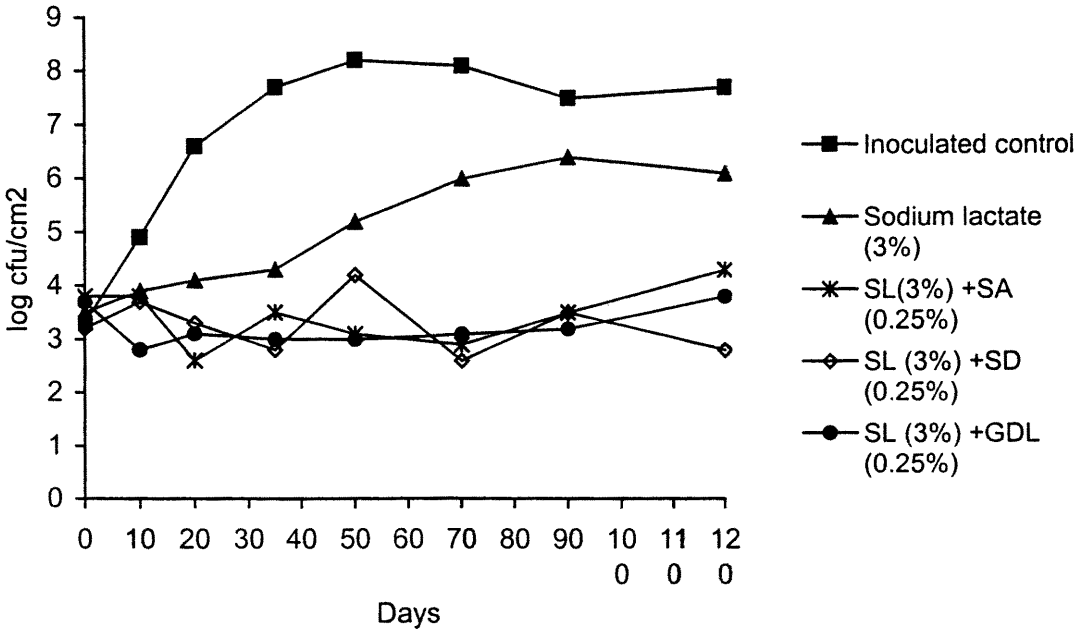

Figure IV.10 (Table Appendix IV 10). Changes (least squares means of log cfu/cm^2, n=6) in bacterial counts (TSAYE agar) on the surface of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (80°C) and stored at 4°C (Standard Deviations vary from 0.1 to 2.9). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

In addition, when sodium lactate (2%) was combined with glucono-delta-lactone (0.25 or 0.5%) in the formulation, growth

Table IV.5. Mean (Standard Deviation) reduction (least squares means of difference of log cfu/cm^2 between not dipped and dipped samples) of the populations of *L. monocytogenes* (on PALCAM agar) inoculated on the surface of vacuum packaged frankfurters with combined antimicrobials, dipped or not dipped in hot (80°C) water for 60 sec.

Treatments	Reduction *of L. monocytogenes* counts by the thermal treatment
Inoculated control	0.59 (0.24)
Sodium lactate (SL, 3%)	0.55 (0.14)
SL (3%) + SA (0.25%)	0.44 (0.10)
SL (3%) + SD (0.25%)	0.53 (0.19)
SL (3%) + GDL (0.25%)	0.96 (0.32)

Means were not significantly different (p>0.05).
SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

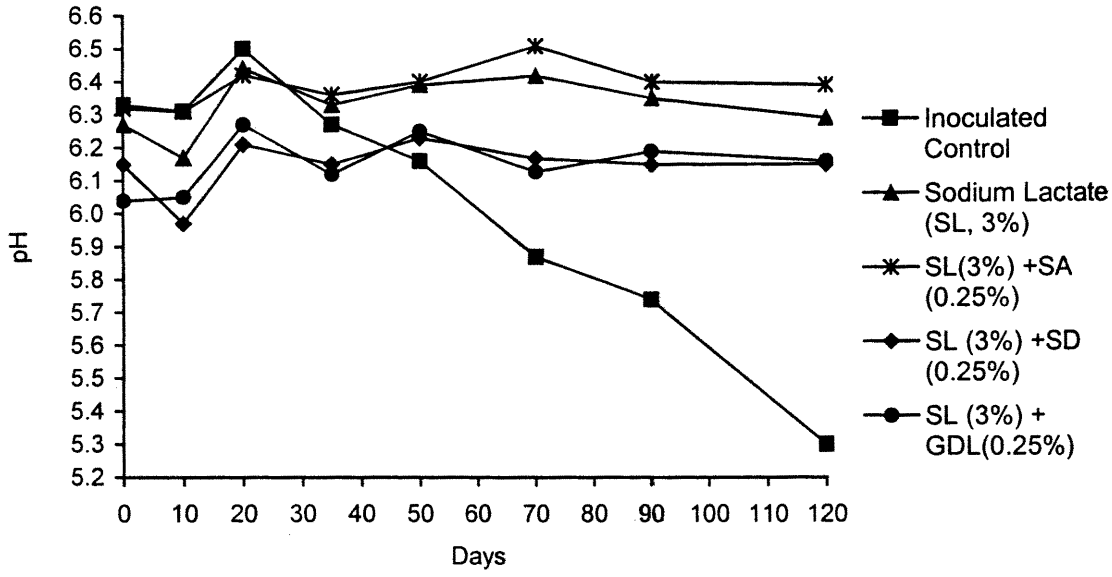

Figure IV.11 (Table Appendix IV 11). Changes in pH (least squares means; n=6) of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, not dipped in hot water, and stored at 4°C for 120 days (Standard Deviations varied from 0.03 to 0.33). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

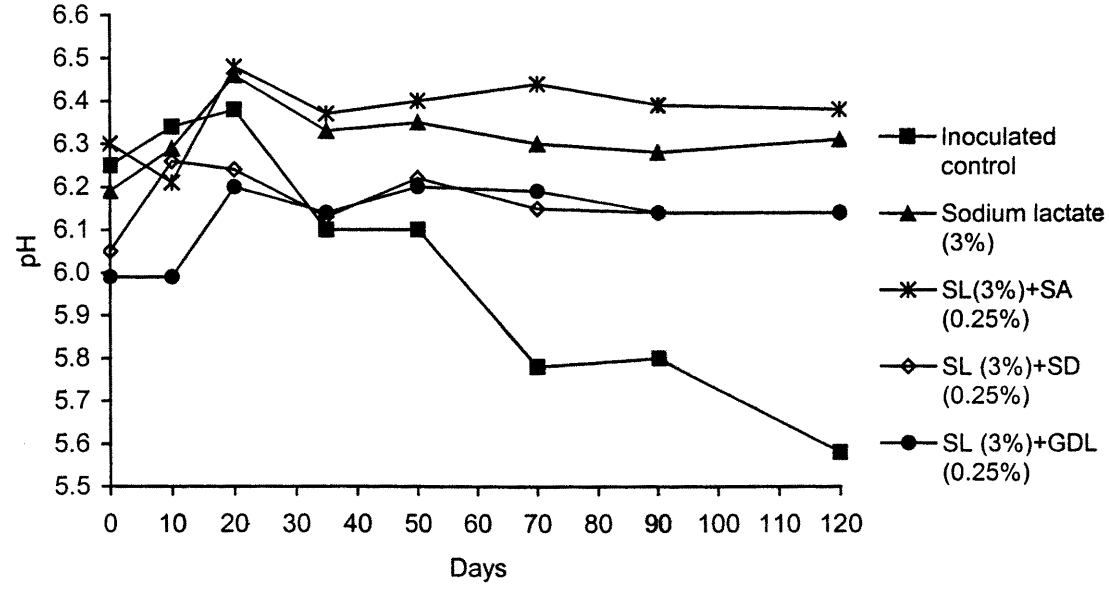

Figure IV.12 (Table Appendix IV 12). Changes in pH (least squares means; n=6) of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (80°C) and stored at 4°C for 120 days (Standard Deviations vary from 0.01 to 0.32). SA: sodium acetate, SD: sodium diacetate, GDL: glucono-delta-lactone.

Table IV.6. Least squares means (SD, n=4) ofcooking yield, water activity (a_w), moisture, and fat contents, before dipping, of frankfurters with combined antimicrobials in the formulation, stored at 4°C.

Treatments	Cooking yield (%)	Water activity (a_w)	Moisture (%)	Fat (%)
Control	93.18	0.965	53.61 y	24.03 z
	(6.45)	(0.00)	(1.01)	(1.31)
Sodium lactate (SL, 3%)	92.74	0.962	52.66 y	24.04 z
	(6.75)	(0.01)	(0.70)	(1.86)
SL(3%)+SA (0.25%)	93.42	0.958	52.40 y	23.77 z
	(5.81)	(0.00)	(1.18)	(1.15)
SL (3%)+SD (0.25%)	92.78	0.957	49.86 z	27.00 y
	(6.68)	(0.00)	(0.30)	(0.63)
SL (3%)+GDL (0.25%)	93.25	0.961	52.88 y	23.65 z
	(6.03)	(0.00)	(1.01)	(0.51)

y z: means within a column lacking a common superscript letter differ ($p<0.05$). Means for water acivity and cooking yield were not significantly different ($p>0.05$).

of the pathogen was suppressed for 35 days at 5 or 10 °C. Research on the effect of chemical hurdles on *L. monocytogenes* (Juncher et al., 2000), indicated that 2.0% sodium lactate combined with 0.25% glucono-delta-lactone (GDL) prevented growth of *L. monocytogenes* inoculated on highly seasoned pork sausage slices prepared with 60 or 150 ppm nitrite, packaged in modified atmosphere (80% N_2/20% CO_2) and stored at 5 or 10°C for 28 days. When sodium lactate (1.8%) was combined with nisin (120-180 IU/g) and injected to the fish before smoking, the growth was inhibited for 29 days at 3°C, showing the synergistic activity of the chemicals.

CONCLUSIONS

When permissible levels of sodium lactate, sodium acetate, sodium diacetate, and glucono-delta-lactone (i.e., 3, 0.25, 0.25, and 0.25%, respectively) were combined and

incorporated in the formulation of frankfurters, growth of *L. monocytogenes* was completely inhibited during storage (120 days) at 4°C. In addition, thermal treatment (dipping in hot water at 80°C) of products containing combinations of additives enhanced the antimicrobials activity of these additives. When sodium lactate was added in the formulation alone, it prevented growth of the pathogen for 35-50 days whether the frankfurters were dipped or not in hot water.

The thermal treatment on surface inoculated *Listeria monocytogenes* on frankfurters with no antimicrobials had no effect against the pathogen except when one frankfurter per bag was vacuum packaged before dipping in hot (75 or 80°C) water. However, the thermal treatment provided an important initial reduction of the pathogen on the frankfurters after dipping.

Combinations of sodium lactate with sodium diacetate or glucono-delta-lactone decreased pH on day 0 while pH for all samples with no antimicrobials decreased dramatically after 35 days. None of the water activity, the cooking yield, the moisture or the fat content was affected by the combined antimicrobials.

CHAPTER V

SUMMARY

The results of these studies indicate that permissible levels of sodium acetate and sodium diacetate inhibited growth of *Listeria monocytogenes* for 20 to 35 days while permissible level of sodium lactate inhibited growth of the pathogen for 50 days. In addition, doubling the permissible levels of sodium lactate and sodium diacetate permitted complete inhibition of the organism during storage (120 days) at refrigerated temperature while increase of the concentration of sodium acetate inhibited growth for 35 days at 4°C. Increases of permissible levels of these antimicrobials could be avoided by combining them in the formulation of frankfurters at currently permitted levels which provided complete inhibition the pathogen for 120 days at 4°C. Thermal treatment was effective in immediate reduction of pathogen counts and it provided an enhanced antimicrobial activity of combinations of antimicrobials on *L. monocytogenes* inoculated on the surface of frankfurters.

Consumer tasting panel and technological testing must be performed prior to an eventual increase of the concentrations of theses antimicrobials in cooked meat product formulations. In addition, more studies on the antimicrobial activity of additives under temperature (>4°C) abusive conditions and on other types of microorganisms may be performed to evaluate product shelf life.

CHAPTER VI

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CHAPTER VII

APPENDIX

TABLES FOR CHAPTER III

Table Appendix III.1 (for Figure III.1). Changes (least squares means of log cfu/cm^2, n=9) in mean (Standard deviations) populations of inoculated *Listeria monocytogenes* (PALCAM agar) on the surface of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculated Control	<0.8 [C]	<0.8	0.9 xA (0.5)	0.9 yA (0.2)	1.1 zA (0.8)	1.5 zA (0.9)	<0.8 [C]	<0.8 [C]
Inoculated Control	3.2 vD (0.4)	4.8 vC (1.1)	6.2 vB (1.3)	7.8 vA (0.5)	8.2 vA (0.4)	8.3 vA (0.2)	8.2 vA (0.1)	8.2 vA (0.2)
Sodium Acetate (0.25%)	3.3 vD (0.7)	3.0 wD (0.5)	3.8 wD (0.8)	5.2 wC (1.5)	6.6 wB (2.0)	7.1 wAB (1.8)	7.6 wAB (0.9)	7.9 vA (0.4)
Sodium Acetate (0.50%)	3.3 vDE (0.6)	2.6 wE (0.1)	3.2 wDE (0.7)	4.2 wxCD (1.2)	4.9 xB (2.1)	5.8 xAB (2.5)	5.8 xAB (2.0)	6.2 wA (2.1)
Sodium Diacetate (0.25%)	3.2 vE (0.5)	2.5 wDE (0.2)	3.3 wCDE (1.4)	3.7 xCD (1.1)	4.4 xBC (1.8)	5.2 xAB (2.5)	5.6 xyAB (2.7)	5.5 wA (2.9)
Sodium Diactate (0.50%)	3.4 vA (0.7)	2.4 wAB (0.1)	2.7 wAB (0.4)	2.6 xAB (0.6)	2.8 yAB (0.6)	1.8 zB (0.8)	1.7 zB (0.8)	1.6 zB (0.6)
Sodium Lactate (3%)	3.2 vBCD (0.6)	2.4 wD (0.7)	2.7wCD (0.5)	2.9 xCD (0.6)	3.5 yBCD (0.7)	3.9 yABC (1.8)	4.5 yA (1.8)	4.1 xAB (2.6)
Sodium Lactate (6%)	3.2 vA (0.7)	2.4 wAB (0.1)	2.7 wAB (0.5)	2.7 xAB (0.6)	2.6 yAB (0.6)	2.4 zAB (0.7)	2.0 zB (1.0)	1.8 zB (1.0)

ABCDE: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
[C]: value below the detection level (no colonies were detected on the agar)

Table Appendix III.2 (for Figure III.2). Changes (least squares means of log cfu/cm^2, n=9) in mean (standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculated	2.3 wB	2.9 wB	1.9 zB	2.8 yB	4.2 xYA	4.4 xA	4.5 xyA	5.3 xyA
Control	(2.2)	(2.3)	(1.3)	(1.7)	(2.1)	(2.5)	(2.8)	(2.5)
Inoculated	4.0 vD	5.3 vC	7.1 vB	7.8 vAB	8.2 uAB	8.4 vA	8.3 vA	8.4 vA
Control	(1.1)	(0.7)	(0.7)	(0.5)	(0.3)	(0.2)	(0.2)	(0.3)
Sodium	4.0 vD	3.9 wD	4.7 wCD	5.3 wC	6.7 vB	7.1wAB	7.7 vAB	7.9 vA
Acetate (0.25%)	(1.0)	(0.7)	(0.4)	(1.2)	(1.9)	(1.8)	(0.8)	(0.4)
Sodium	4.1 vD	3.5 wD	4.0 wxD	4.5 xCD	5.5 BC	6.2 AB	6.2 wAB	6.7 wA
Acetate (0.50%)	(1.1)	(1.0)	(0.6)	(0.7)	(1.4)	(2.0)	(1.7)	(1.5)
Sodium	3.6 vC	3.3 wC	3.1 xyC	4.1 xBC	4.7 wB	6.0 wA	6.0 wA	6.5 wA
Diacetate (0.25%)	(0.6)	(0.9)	(1.5)	(0.7)	(1.2)	(1.5)	(2.2)	(1.4)
Sodium	4.0 vA	2.5 CD	2.9 yzBCD	2.5 yCD	2.4 zD	2.5 yCD	3.5 ABC	4.3 yzA
Diactate (0.50%)	(0.6)	(1.1)	(0.8)	(1.0)	(1.1)	(0.9)	(0.7)	(0.9)
Sodium	3.7 vBCD	3.1 CD	3.3 xCD	3.2 yCD	3.0 yD	4.3 xBC	4.8 xAB	5.8 wxA
Lactate (3%)	(0.7)	(0.7)	(0.9)	(0.6)	(0.5)	(0.3)	(1.6)	(1.8)
Sodium	3.3 vwAB	2.7 AB	2.2 yzB	2.9 yAB	3.1yAB	3.6 xyA	3.7 xyA	3.7 zA
Lactate (6%)	(0.6)	(0.5)	(1.0)	(0.6)	(0.5)	(0.8)	(0.8)	(1.6)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
uvwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix III.3 (for Figure III.3) Changes (least squares means of pH, n=9) in mean (Standard Deviations) pH values of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculated	6.32 yAB	6.22 yB	6.28 AB	6.37 wAB	6.30 vAB	6.36 wAB	6.25 xAB	6.40 wA
Control	(0.16)	(0.04)	(0.12)	(0.09)	(0.22)	(0.25)	(0.42)	(0.44)
Inoculated	6.33 yA	6.21 yA	6.24 yA	6.18 xyA	5.61 uBC	5.62 zB	5.46 zCD	5.42 zD
Control	(0.09)	(0.02)	(0.05)	(0.38)	(0.24)	(0.26)	(0.21)	(0.28)
Sodium	6.35 yAB	6.22 yBC	6.16 yzC	6.41 wA	6.15 wC	6.19 xC	5.86 yD	5.92 yD
Acetate (0.25%)	(0.12)	(0.02)	(0.04)	(0.09)	(0.15)	(0.20)	(0.33)	(0.18)
Sodium	6.36 ABC	6.23 yC	6.23 yC	6.39 wAB	6.31 ABC	6.43 wA	6.33 xC	6.29 wBC
Acetate (0.50%)	(0.13)	(0.04)	(0.11)	(0.06)	(0.14)	(0.09)	(0.19)	(0.20)
Sodium	6.03 zBCD	5.95 zD	5.98 zCD	6.10 yABCD	6.11 ABC	6.17 xAB	6.25 xA	6.13 xAB
Diacetate (0.25%)	(0.10)	(0.07)	(0.13)	(0.05)	(0.20)	(0.14)	(0.09)	(0.28)
Sodium	5.87 zBC	5.83 zBC	5.70 zD	5.94 zABC	5.94 ABC	6.01 yAB	6.00 yAB	6.02 xyA
Diactate (0.50%)	(0.07)	(0.04)	(0.06)	(0.10)	(0.17)	(0.12)	(0.13)	(0.26)
Sodium	6.23 yB	6.27 yAB	6.20 yB	6.33 wxAB	6.31 uAB	6.38 wA	6.38 xA	6.33 wAB
Lactate (3%)	(0.07)	(0.02)	(0.08)	(0.08)	(0.12)	(0.10)	(0.09)	(0.15)
Sodium	6.31 ABC	6.22 yBC	6.16 yzC	6.34 wAB	6.30 ABC	6.40 wA	6.39 xA	6.37 wAB
Lactate (6%)	(0.07)	(0.09)	(0.03)	(0.12)	(0.16)	(0.10)	(0.13)	(0.16)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
uvwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix III.4 (for Figure III.4). Changes (least squares means of log cfu/cm^2, n=3) in means (Standard Deviation) population of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with antimicrobials in the formulation, vacuum packaged and stored at 4°C

Treatments	Storage at 4oC (days)				
	0	10	20	35	50
Uninoculated control	<0.8 [C]	<0.8 [C]	0.8 zA (0.0)	1.0 zA (0.3)	1.1 zA (0.6)
Inoculated control	4.0 xyD (0.2)	4.7 xC (0.1)	6.0 wB (0.4)	8.0 wA (0.7)	8.4 Xa (0.2)
Nisin (0.15%)	3.3 zD (0.4)	3.0 zC (0.1)	4.6 yB (0.2)	4.5 yB (0.3)	7.6 yA (0.1)
Allyl isothiocyanate (0.02%)	4.1 xE (0.3)	4.8 wxD (0.3)	5.7 wxC (0.3)	7.4 xB (0.3)	8.2 xA (0.2)
Lysozyme (0.01%)	3.8 yzE (0.4)	4.9 wxD (0.3)	5.7 wxC (0.2)	7.2 xB (0.2)	8.1 xyA (0.3)
Monolaurin (0.01%)	4.0 xyD (0.2)	5.3 wC (0.3)	7.1 vB (0.3)	8.0 wA (0.4)	8.3 xA (0.1)
Gluco-nodelta-lactone (0.25%)	4.0 xyC (0.1)	4.2 Yc (0.4)	5.3 xB (0.2)	7.6 wxA (0.3)	7.9 xyA (0.3)

ABCDE: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
[C]: value below the detection level (no colony on the agar)

Table Appendix III.5 (for Figure III.5). Changes (least squares means of log cfu/cm^2, n=3) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C

Treatments	Storage at 4°C (days)				
	0	10	20	35	50
Uninoculated control	1.0 Zb	1.0 zB	1.3 zB	1.3 zB	3.0 zA
	(0.1)	(0.4)	(0.7)	(0.7)	(0.5)
Inoculated control	4.2 yC	4.8 xyC	6.2 xB	8.4 wA	8.0 yA
	(0.1)	(0.1)	(0.2)	(0.3)	(0.7)
Nisin (0.15%)	4.0 yB	4.1 yB	4.8 yB	4.7 yB	7.6 yA
	(0.1)	(0.5)	(0.1)	(0.1)	(0.1)
Allyl isothiocyanate (0.02%)	4.4 yD	4.9 xyD	5.7 xyC	7.6 wxB	8.2 yA
	(0.1)	(0.3)	(0.3)	(0.5)	(0.2)
Lysozyme (0.01%)	4.2 yD	4.9 xyD	5.8 xyC	7.3 xB	8.2 yA
	(0.2)	(0.2)	(0.3)	(0.1)	(0.2)
Monolaurin (0.01%)	4.2 yD	5.3 xC	7.2 wB	7.9 wxAB	8.3 yA
	(0.0)	(0.3)	(0.3)	(0.5)	(0.1)
Gluco-nodelta-lactone (0.25%)	4.3 yC	4.2 yC	5.4 yB	7.7 wxA	8.0 yA
	(0.2)	(0.3)	(0.2)	(0.4)	(0.3)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix III.6 (for Figure III.6). Changes (least squares means of pH, n=3) in mean (Satndard Deviations) pH values of frankfurters with antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged and stored at 4°C

Treatments	Storage at 4°C (days)				
	0	10	20	35	50
Uninoculated control	6.35 zA	6.35 yzA	6.37 yzA	6.34 yA	6.42 xA
	(0.03)	(0.09)	(0.08)	(0.05)	(0.00)
Inoculated control	6.30 zB	6.31yzB	6.57 wxA	6.34 yAB	5.92 yzC
	(0.06)	(0.11)	(0.07)	(0.51)	(0.21)
Nisin (0.15%)	6.32 zAB	6.45 yAB	6.50 xyA	6.03 zC	6.29 xB
	(0.07)	(0.04)	(0.10)	(0.03)	(0.03)
Allyl isothiocyanate (0.02%)	6.24 zAB	6.35 yzA	6.29 zAB	6.15 yzBC	6.04 yC
	(0.09)	(0.07)	(0.06)	(0.03)	(0.14)
Lysozyme (0.01%)	6.26 zBC	6.39 yzB	6.60 wA	6.05 zD	6.09 yCD
	(0.06)	(0.03)	(0.08)	(0.08)	(0.11)
Monolaurin (0.01%)	6.35 zA	6.27 yzA	6.34 yzA	6.03 zB	5.75 zC
	(0.05)	(0.08)	(0.09)	(0.10)	(0.10)
Glucono-delta-lactone (0.25%)	6.14 zAB	6.12 zAB	6.24 zA	5.97 zB	5.95 yB
	(0.13)	(0.12)	(0.07)	(0.08)	(0.24)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

TABLES FOR CHAPTER IV

Table Appendix IV.1 (for Figure IV.1) Changes (least squares means of log cfu/cm^2, n=3) in mean (Standard Deviations) populations of inoculated *Listeria monocytogenes* (PALCAM agar) on the surface of frankfurters without antimicrobial in the formulation, vacuum packaged, dipped in hot water (75°C), and stored at 4°C for 50 days.

Treatments	Storage at 4°C (days)				
	0	10	20	35	50
Uninoculated control not dipped 1/bag	0.8 zA (0.0)	<0.8 [C]	<0.8 [C]	<0.8 [C]	1.0 zA (0.3)
Uninoculated control not dipped 2/bag	0.8 zA (0.0)	<0.8 [C]	0.8 zA (0.0)	1.2 zA (0.7)	0.8 zA (0.0)
Inoculated control not dipped 1/bag	3.9 wC (0.1)	4.2 xC (0.2)	5.3 wxB (0.4)	7.6 vwA (0.4)	8.4 wA (0.0)
Inoculated control not dipped 2/bag	3.9 wC (0.0)	4.0 xC (0.2)	5.7 wB (0.6)	8.3 vA (0.2)	8.6 wA (0.1)
Inoculated dipped 30s 1/bag	1.9 yC (0.3)	2.8 yB (0.0)	3.2 yB (0.5)	5.6 yA (0.3)	6.4 xA (0.6)
Inoculated dipped 30s 2/bag	3.0 xD (0.1)	3.5 xyD (0.2)	5.8 wC (0.1)	7.4 wxB (0.2)	8.4 wA (0.0)
Inoculated dipped 60s 1/bag	0.8 zD (0.1)	0.9 zD (0.2)	2.9 yC (0.6)	5.7 yB (0.3)	7.0 xA (1.5)
Inoculated dipped 60s 2/bag	2.8 xD (0.2)	3.1 yD (0.3)	4.6 xC (0.3)	6.9 wxB (0.2)	8.3 wA (0.1)
Inoculated dipped 90s 1/bag	0.8 zB (0.0)	1.6 zAB (1.4)	<0.8	1.2 zB (0.8)	2.1 yA (1.5)
Inoculated dipped 90s 2/bag	2.8 xD (0.4)	2.9 yD (0.1)	4.5 xC (0.1)	6.6 xB (0.1)	8.3 wA (0.2)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
[C]: value below the detection level (no colony on the agar)

Table Appendix IV.2 (for Figure IV.2). Changes (least squares means of log cfu/cm^2, n=3) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters without antimicrobial in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (75°C) and stored at 4°C for 50 days.

Treatments	Storage at 4°C (days)				
	0	10	20	35	50
Uninoculated control not dipped 1/bag	1.7 xyB (0.5)	1.1 yzB (0.1)	1.0 zB (0.3)	3.7 yA (0.1)	1.3 zB (0.5)
Uninoculated control not dipped 2/bag	0.8 zA (0.0)	0.8 zA (0.1)	0.8 zA (0.0)	1.7 zA (1.5)	0.9 zA (1.2)
Inoculated control not dipped 1/bag	3.9 vC (0.1)	4.1 wC (0.1)	5.5 vB (0.8)	8.0 uwA (0.4)	8.4 wA (0.0)
Inoculated control not dipped 2/bag	3.9 vC (0.0)	4.2 wC (0.2)	6.0 vB (0.1)	8.3 uA (0.2)	8.6 wA (0.1)
Inoculated dipped 30s 1/bag	2.5 wxC (0.2)	2.9 xC (0.1)	2.6 yC (0.6)	5.6 xB (0.3)	6.6 xA (1.1)
Inoculated dipped 30s 2/bag	3.2 vwC (0.1)	3.5 wxC (0.2)	5.7 vB (0.2)	7.4 vwA (0.1)	8.3 wA (0.0)
Inoculated dipped 60s 1/bag	1.4 yzD (0.5)	1.3 yzD (0.3)	2.9 xyC (0.6)	5.6 xB (0.2)	6.9 xA (1.5)
Inoculated dipped 60s 2/bag	3.0 wC (0.2)	3.2 xC (0.3)	3.8 wxC (1.4)	7.0 vwB (0.3)	8.3 wA (0.1)
Inoculated dipped 90s 1/bag	1.0 yzBC (0.2)	1.8 yAB (1.3)	0.8 zC (0.0)	1.3 zBC (0.7)	2.4 yA (2.0)
Inoculated dipped 90s 2/bag	2.9 wD (0.3)	3.0 xD (0.2)	4.3 wC (0.6)	6.7 wB (0.2)	8.2 wA (0.2)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
uvwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.3 (for Figure IV.3). Changes (least squares means of pH, n=3) in mean (Standard Deviations) pH values of frankfurters without antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged dipped in hot water (75°C), and stored at 4°C for 50 days.

Treatments	Storage at 4°C (days)				
	0	10	20	35	50
Uninoculated control not dipped 1/bag	6.34 xyA (0.07)	6.30 yzAB (0.02)	6.19 zB (0.04)	6.33 yzA (0.04)	6.26 wAB (0.02)
Uninoculated control not dipped 2/bag	6.48 wA (0.02)	6.38 yzAB (0.03)	6.24 yzC (0.04)	6.45 xyA (0.04)	6.34 wBC (0.02)
Inoculated control not dipped 1/bag	6.26 yzA (0.03)	6.29 yzA (0.01)	6.19 zA (0.02)	6.26 zA (0.01)	5.83 yB (0.07)
Inoculated control not dipped 2/bag	6.41 wxA (0.03)	6.39 yzB (0.02)	6.25 yzC (0.02)	6.64 wA (0.47)	5.64 zD (0.12)
Inoculated dipped 30s 1/bag	6.15 zB (0.01)	6.32 yzA (0.01)	6.23 yzAB (0.04)	6.36 xyzA (0.02)	6.27 wAB (0.04)
Inoculated dipped 30s 2/bag	6.21 yzBC (0.06)	6.26 zAB (0.04)	6.23 yzB (0.01)	6.39 xyzA (0.07)	6.09 xC (0.04)
Inoculated dipped 60s 1/bag	6.25 yzB (0.11)	6.32 yzAB (0.02)	6.27 yzB (0.03)	6.40 xyA (0.05)	6.29 wB (0.08)
Inoculated dipped 60s 2/bag	6.25 yzB (0.09)	6.38 yzAB (0.01)	6.30 yAB (0.02)	6.41 xyA (0.03)	6.25 wB (0.03)
Inoculated dipped 90s 1/bag	6.22 yzAB (0.05)	6.28 yzAB (0.02)	6.19 zB (0.01)	6.35 yzA (0.04)	6.35 wA (0.04)
Inoculated dipped 90s 2/bag	6.44 wxA (0.03)	6.40 yA (0.03)	6.25 yzB (0.03)	6.49 xA (0.03)	6.22 wB (0.13)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.4 (for Figure IV.4) Changes (least squares means of log CFU /cm^2, n=3) in mean (Standard Deviations) populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters without antimicrobial in the formulation, vacuum packaged, dipped in hot water (80°C), and stored at 4°C for 70 days.

Treatments	Storage at 4°C (days)					
	0	10	20	35	50	70
Uninoculated control not dipped 1/bag	<0.8 [C]	1.0 zA (0.2)	<0.8 [C]	1.1 zA (0.6)	0.8 zA (0.1)	<0.8 [C]
Uninoculated control not dipped 2/bag	0.8 zA (0.0)	0.8 zA (0.0)	<0.8 [C]	<0.8 [C]	0.8 zA (0.0)	<0.8 [C]
Inoculated control not dipped 1/bag	4.0 wCD (0.0)	3.7 wxD (0.1)	5.1 vwxBC (0.7)	6.3 vwAB (0.8)	6.5 xA (1.1)	7.3 yA (0.4)
Inoculated control not dipped 2/bag	4.0 wC (0.1)	4.4 wC (0.2)	6.1 vB (0.1)	7.4 vA (0.1)	6.6 xAB (0.3)	7.0 yAB (0.5)
Inoculated dipped 30s 1/bag	2.8 yD (0.2)	2.5 xyD (0.2)	3.9 xyC (0.5)	6.1 vwB (1.2)	7.0 xAB (0.9)	7.7 yA (0.4)
Inoculated dipped 30s 2/bag	3.7 wxC (0.1)	4.2 wC (0.6)	5.5 vwB (0.3)	6.3 vwAB (0.5)	6.5 xAB (0.8)	7.1 yA (0.5)
Inoculated dipped 60s 1/bag	3.1 wxyCD (0.3)	2.0 yzD (0.2)	3.3 yC (0.6)	4.6 xyB (1.1)	6.8 xA (1.0)	7.9 yA -
Inoculated dipped 60s 2/bag	3.5 wxyC (0.1)	3.4 wxC (0.6)	4.9 wxB (0.2)	5.8 wxAB (0.4)	6.7 xA (0.8)	6.4 yA (1.0)
Inoculated dipped 90s 1/bag	2.8 xyBC (0.3)	2.0 yzC (1.1)	1.6 zC (1.4)	4.0 yA (1.8)	3.4 yAB (2.7)	1.7 zC (1.3)
Inoculated dipped 90s 2/bag	3.2 wxyC (0.1)	2.6 xyC (0.1)	4.7 wxB (0.6)	5.5 wxB (0.5)	6.9 xA (0.6)	6.8 yA (0.3)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
[C]: value below the detection level (no colony on the agar)
On day 70, inoculated dipped 60s 1/bag was represented by one sample.

Table Appendix IV.5 (for Figure IV.5). Changes (least squares means of log cfu/cm^2, n=3) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters without antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged dipped in hot water (80°C), and stored at 4°C for 70 days.

Treatments	Storage at 4°C (days)					
	0	10	20	35	50	70
Uninoculated control	3.7 xyzC	6.0 wB	5.2 xB	6.6 xyAB	7.8 yzA	7.5 zA
not dipped 1/bag	(1.9)	(1.6)	(0.5)	(1.6)	(0.5)	(0.3)
Uninoculated control	2.5 zC	4.7 xyB	7.2 vA	7.3 vwxA	7.8 yzA	8.1 zA
not dipped 2/bag	(0.5)	(1.5)	(0.1)	(0.6)	(0.1)	(0.2)
Inoculated control	4.5 xbC	3.5 yzC	5.6 wxB	7.9 vwA	8.5 yA	8.2 zA
not dipped 1/bag	(0.4)	(0.3)	(1.0)	(0.4)	(0.2)	(0.4)
Inoculated control	4.4 xC	4.7 xyC	6.5 vwB	8.6 vA	8.2 yA	8.0 zA
not dipped 2/bag	(0.4)	(0.4)	(0.3)	(0.4)	(0.1)	(0.2)
Inoculated dipped	2.9 yzD	4.0 xyD	6.6 vwBC	5.6 yzC	7.8 yzAB	8.0 zA
30s 1/bag	(0.2)	(1.1)	(0.1)	(1.6)	(0.5)	(0.1)
Inoculated dipped	4.4 xB	4.9 wxB	6.8 vwA	7.2 wxA	7.8 yzA	7.8 zA
30s 2/bag	(0.6)	(1.1)	(0.4)	(0.5)	(0.6)	(0.3)
Inoculated dipped	3.9 xyB	4.0 xyB	3.8 yB	5.0 zB	7.9 yzA	7.8 zA
60s 1/bag	(1.0)	(2.0)	(1.0)	(0.8)	(0.4)	(0.4)
Inoculated dipped	4.3 xD	4.4 xyD	5.9 wxC	7.0 wxAB	7.6 yzA	7.5 zA
60s 2/bag	(0.6)	(1.8)	(0.4)	(0.5)	(0.3)	(0.1)
Inoculated dipped	4.0 xyB	2.7 zC	2.5 zC	4.7 zB	6.9 zA	7.4 zA
90s 1/bag	(1.0)	(0.3)	(1.1)	(2.3)	(0.3)	(0.4)
Inoculated dipped	4.3 xC	2.9 zD	4.9 xyC	6.2 xyB	7.4 yzA	7.1zAB
90s 2/bag	(0.9)	(0.2)	(0.7)	(0.3)	(0.3)	(0.2)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.6 (for Figure IV.6). Changes (least squares means of pH, n=3) in mean (Standard Deviations) pH values of frankfurters without antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (80°C), and stored at 4°C for 70 days.

Treatments	Storage at 4°C (days)					
	0	10	20	35	50	70
Uninoculated control not dipped 1/bag	6.16 zA (0.07)	6.17 zA (0.02)	6.11 zA (0.12)	6.17 yzA (0.41)	6.22 vA (0.15)	6.14 wA (0.03)
Uninoculated control not dipped 2/bag	6.29 zA (0.07)	6.34 zA (0.04)	6.13 zAB (0.09)	6.41 yA (0.04)	5.85 vwxBC (0.30)	5.52 xyzC (0.44)
Inoculated control not dipped 1/bag	6.20 zA (0.05)	6.23 zA (0.03)	6.12 zA (0.06)	5.97 zAB (0.40)	5.41 yzC (0.49)	5.73 xyBC (0.38)
Inoculated control not dipped 2/bag	6.36 zA (0.11)	6.37 zA (0.04)	6.38 zA (0.02)	6.24 yzA (0.07)	5.22 zB (0.20)	5.44 yzB (0.38)
Inoculated dipped 30s 1/bag	6.36 zA (0.12)	6.19 zA (0.03)	6.07 zA (0.21)	6.33 yA (0.08)	5.59 xyzB (0.66)	5.71 xyB (0.39)
Inoculated dipped 30s 2/bag	6.23 zA (0.02)	6.38 zA (0.09)	6.25 zA (0.09)	6.42 yA (0.10)	5.73 wxyB (0.47)	5.44 yzB (0.26)
Inoculated dipped 60s 1/bag	6.15 zA (0.02)	6.24 zA (0.02)	6.25 zA (0.04)	6.44 yA (0.06)	5.60 xyxB (0.63)	5.26 zB (0.62)
Inoculated dipped 60s 2/bag	6.37 zA (0.07)	6.38 zA (0.14)	6.33 zA (0.12)	6.20 yzAB (0.28)	5.88 vwxB (0.12)	5.28 zB (0.12)
Inoculated dipped 90s 1/bag	6.38 zA (0.13)	6.29 zA (0.03)	6.24 zA (0.03)	6.33 yA (0.14)	6.05 vwA (0.03)	6.16 wA (0.05)
Inoculated dipped 90s 2/bag	6.41 zA (0.05)	6.44 zA (0.06)	6.34 zA (0.01)	6.44 yA (0.08)	6.07 vwAB (0.01)	5.89 wxB (0.22)

ABC: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.7 (for Figure IV.7) Changes (least squares means of log CFU /cm^2, n=6) in mean (Standard deviations) populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with combined antimicrobials in the formulation, vacuum packaged, not dipped in hot water, and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculum	<0.8 [C]	<0.8 [C]	1.0 zA	0.8 zA	0.9 zA	1.2 zA	2.1 z	0.8 zA
control	(0.0)	(0.0)	(0.4)	(0.1)	(0.5)	(0.7)	(1.7)	(0.0)
Inoculated	3.1zC	3.8 yC	6.0 xB	7.2 xA	6.9 wAB	7.8 wA	7.8 xA	7.5 xA
control	(0.1)	(1.3)	(1.4)	(0.9)	(0.7)	(0.3)	(0.3)	(0.6)
Sodium	3.2 zC	2.8 zC	2.9 yC	3.1 yC	5.5 xB	6.7 xA	6.2 yAB	6.3 yAB
lactate (3%)	(0.2)	(0.2)	(0.4)	(1.7)	(1.0)	(0.4)	(1.0)	(1.1)
SL(3%)	3.1 zA	2.7 zAB	2.6 yABC	2.3 yABC	1.6 yC	2.2 yzABC	2.3 zABC	1.7 zBC
+SA (0.25%)	(0.1)	(0.1)	(0.1)	(0.4)	(0.6)	(0.2)	(0.4)	(0.4)
SL (3%)	3.2 zA	2.6 zAB	2.6 yAB	2.2 yBC	1.6 yC	2.0 yzBC	1.9 zBC	1.6 zC
+SD (0.25%)	(0.2)	(0.1)	(0.0)	(0.3)	(0.6)	(0.2)	(0.4)	(0.2)
SL (3%)	3.5 zA	2.6 zAB	2.6 yAB	2.2 yB	1.6 yB	2.3 yBB	2.2 zBB	2.0 zB
+GDL (0.25%)	(0.3)	(0.1)	(0.1)	(0.5)	(0.6)	(0.4)	(0.2)	(0.2)

ABC: means within a row lacking a common superscript letter differ ($p<0.05$)

wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

[C]: value below the detection level (no colony on the agar)

Table Appendix IV.8 (for Figure IV.8) Changes (least squares means of log CFU /cm^2 ± SD n=6) in mean (Standard Deviation) populations of inoculated *L. monocytogenes* (PALCAM agar) on the surface of frankfurters with combined antimicrobials in the formulation, vacuum packaged, dipped in hot water (80^{0}C) and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculated control	1.0 zA (0.2)	<0.8 [C]	0.9 zA (0.3)	<0.8 [C]	1.2 zA (0.7)	<0.8 [C]	1.4 zA (0.9)	<0.8 [C]
Inoculated control	2.6 yD (0.1)	4.5 yC (1.9)	6.2 xB (2.2)	7.6 wA (1.0)	7.8 xA (0.8)	7.9 xA (0.4)	7.5 xA (0.2)	7.6 wA (0.1)
Sodium lactate (3%)	2.6 yDE (0.2)	2.3 zE (0.6)	3.5 xCD (2.0)	3.8 xC (1.7)	4.4 yBC (1.6)	5.3 yAB (2.5)	5.4 yAB (2.3)	6.1 xA (1.5)
SL(3%)+ SA (0.25%)	2.7 yAB (0.2)	2.2 zB (0.1)	2.0 zB (0.4)	2.3 xyAB (0.4)	1.8 zB (0.6)	1.8 zB (0.5)	2.2 zB (1.6)	3.6 yA (1.3)
SL (3%)+ SD (0.25%)	2.6 yA (0.0)	2.0 zAB (0.4)	2.0 zAB (0.3)	1.9 zAB (0.7)	1.5 zAB (0.4)	1.3 zB (0.3)	1.1 zB (0.5)	0.9 zB (0.3)
SL (3%)+ GDL (0.25%)	2.6 yA (0.1)	2.0 zAB (0.3)	2.1 zAB (0.1)	2.1 yzAB (0.4)	1.5 zAB (0.4)	1.2 zB (0.3)	1.1 zB (0.4)	1.6 zAB (1.0)

ABCDE: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)
[C]: value below the detection level (no colony on the agar)

Table Appendix IV.9 (for Figure IV.9). Changes (least squares means of log cfu/cm^2, n=3) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogene*, vacuum packaged, not dipped in hot water (80°C), and stored at 4°C

Treatments	Storage at 4°C (days							
	0	10	20	35	50	70	90	120
Uninoculum	2.6 zC	2.2 zC	2.1 zC	2.6 zC	4.0 yzAB	4.4yA	4.3 yAB	2.7 zBC
control	(1.4)	(1.2)	(1.8)	(2.4)	(1.4)	(2.2)	(3.2)	(0.0)
Inoculated	3.2 yzC	4.2 xC	6.0 xB	7.4 xA	7.4 wA	8.2 wA	7.7 wA	8.3 xA
control	(1.3)	(1.1)	(1.4)	(0.7)	(0.5)	(0.4)	(0.3)	(0.1)
Sodium	4.1 xyB	3.5 xyBC	3.1 yzC	4.0 yBC	6.1 xA	6.6 xA	6.3 xA	6.5 yA
lactate (3%)	(0.9)	(0.5)	(0.4)	(1.0)	(0.6)	(0.4)	(0.9)	(1.1)
SL(3%)	4.5 xA	3.6 xyABC	3.7 yAB	3.0 zBC	4.2 yA	3.0 zBC	3.0 zBC	2.7 zC
+SA (0.25%)	(1.3)	(1.0)	(1.0)	(0.4)	(1.6)	(0.7)	(0.9)	(0.8)
SL (3%)	3.8 xyA	3.6 xyA	3.5 yA	3.0 zA	3.2 zA	2.8 zA	2.9 zA	3.4 zA
+SD (0.25%)	(0.8)	(0.7)	(0.9)	(0.4)	(1.4)	(0.4)	(0.9)	(1.3)
SL (3%)	3.8 xyA	2.8 yzAB	2.9 yzAB	2.9 zAB	3.8 yzA	3.1 zAB	2.4 zB	2.8 zAB
+GDL (0.25%)	(0.7)	(0.3)	(0.4)	(0.5)	(1.7)	(0.5)	(0.4)	(0.7)

ABC: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.10 (for Figure IV.1). Changes (least squares means of log cfu /cm^2; n=6) in mean (Standard Deviations) populations of bacterial counts (TSAYE agar) on the surface of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (80^{0}C) and stored at 4^{o}C

Treatments	Storage at 4^{o}C (days)							
	0	10	20	35	50	70	90	120
Uninoculum control	3.1zB (1.4)	3.1 zB (1.1)	1.8 zC (0.7)	3.2 yzB (1.2)	5.3 xA (2.9)	4.3 yAB (1.8)	5.1 yA (1.6)	4.0 yzAB (0.81)
Inoculated control	3.3 zD (0.9)	4.9 yC (1.8)	6.6 wB (1.9)	7.7 xAB (1.0)	8.2 wA (0.5)	8.1 wA (0.1)	7.5 xAB (0.2)	7.7 wAB (0.13)
Sodium lactate (3%)	3.5 zC (0.8)	3.9 yzC (0.7)	4.1 xBC (1.7)	4.3 yBC (1.4)	5.2 xAB (1.3)	6.0 xA (1.6)	6.4 xA (1.4)	6.1 xA (1.45)
SL(3%)+SA (0.25%)	3.8 zAB (0.8)	3.8 yzAB (0.9)	2.6 yzB (0.5)	3.5 yzAB (0.6)	3.1 yzAB (0.7)	2.9 zB (0.5)	3.5 zAB (1.2)	4.3 yA (0.27)
SL (3%)+SD (0.25%)	3.2 zAB (0.5)	3.7 yzAB (0.4)	3.3 xyAB (0.9)	2.8 zB (0.9)	4.2 xyA (1.9)	2.6 zB (0.3)	3.5 zAB (1.5)	2.8 zB (1.03)
SL (3%)+GDL (0.25%)	3.7 zA (0.7)	2.8 zA (0.4)	3.1 xyA (0.8)	3.0 zA (0.4)	3.0 zA (0.7)	3.1 zA (1.3)	3.2 zA (1.1)	3.8 yzA (1.29)

AB: means within a row lacking a common superscript letter differ ($p<0.05$)
wxyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.11 (for Figure IV.11). Changes (least squares means of pH, n=6) in mean (Standard Deviation) pH values of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, not dipped in hot water, and stored at 4°C

Treatments	Storage at 4°C (days)							
	0	10	20	35	50	70	90	120
Uninoculum Control	6.34 xA (0.07)	6.28 yA (0.07)	6.47 yA (0.06)	6.34 zA (0.13)	6.46 yA (0.07)	6.40 xyA (0.17)	6.32 yA (0.15)	6.42 yA (0.05)
Inoculated Control	6.33 xAB (0.05)	6.31 yAB (0.04)	6.50 yA (0.04)	6.27 zAB (0.11)	6.16 zB (0.33)	5.87 zC (0.17)	5.74 zC (0.25)	5.30 zC (0.06)
Sodium Lactate (3%)	6.27 yB (0.05)	6.17 yB (0.11)	6.44 yzA (0.13)	6.33 zAB (0.04)	6.39 yzAB (0.10)	6.42 xA (0.14)	6.35 yAB (0.05)	6.29 yAB (0.05)
SL(3%) +SA (0.25%)	6.32 xA (0.03)	6.31 yA (0.06)	6.42 yzA (0.10)	6.36 zA (0.05)	6.40 yzA (0.09)	6.51 xA (0.11)	6.40 yA (0.01)	6.39 yA (0.07)
SL (3%) +SD (0.25%)	6.15 zAB (0.12)	5.97 zB (0.12)	6.21 zAB (0.05)	6.15 zAB (0.04)	6.23 yzA (0.03)	6.17 yAB (0.13)	6.15 yAB (0.06)	6.15 yAB (0.06)
SL (3%) +GDL (0.25%)	6.04 zA (0.06)	6.05 yA (0.06)	6.27 yzA (0.12)	6.12 zA (0.05)	6.25 yzA (0.10)	6.13 yA (0.09)	6.19 yA (0.06)	6.16 yA (0.06)

ABC: means within a row lacking a common superscript letter differ ($p<0.05$)
xyz: means within a column lacking a common superscript letter differ ($p<0.05$)

Table Appendix IV.12 (for Figure IV.12). Changes (least squares means of pH, n=6) in mean (Standard Deviation) pH values of frankfurters with combined antimicrobials in the formulation, inoculated with *L. monocytogenes*, vacuum packaged, dipped in hot water (80^0C) and stored at 4^oC

Treatments	Storage at 4^oC (days)							
	0	10	20	35	50	70	90	120
Uninoculum control	6.21yB (0.05)	6.37 xA (0.04)	6.45 yA (0.06)	6.40 yA (0.05)	6.41 yA (0.07)	6.39 vwA (0.18)	6.37 xA (0.05)	6.38 xA (0.04)
Inoculated control	6.25 yA (0.07)	6.34 xA (0.04)	6.38 yA (0.09)	6.10 zB (0.22)	6.10 zB (0.32)	5.78 zC (0.12)	5.80 zC (0.23)	5.58 zD (0.10)
Sodium lactate (3%)	6.19 yC (0.06)	6.29 xyBC (0.10)	6.46 yA (0.04)	6.33 yBC (0.05)	6.35 yAB (0.08)	6.30 wxBC (0.08)	6.28 xBC (0.10)	6.31 xBC (0.03)
SL(3%)+SA (0.25%)	6.30 yBC (0.03)	6.21 yC (0.14)	6.48 yA (0.05)	6.37 yAB (0.03)	6.40 yAB (0.06)	6.44 vA (0.13)	6.39 xAB (0.01)	6.38 xAB (0.03)
SL (3%)+SD (0.25%)	6.05 zC (0.03)	6.26 xyA (0.18)	6.24 zAB (0.03)	6.13 zBC (0.05)	6.22 zAB (0.07)	6.15 yABC (0.10)	6.14 yBC (0.05)	6.14 yBC (0.05)
SL (3%)+GDL (0.25%)	5.99 zB (0.05)	5.99 zB (0.05)	6.20 zA (0.06)	6.14 zA (0.04)	6.20 zA (0.05)	6.19 xyA (0.09)	6.14 yA (0.04)	6.14 yA (0.04)

ABCD: means within a row lacking a common superscript letter differ ($p<0.05$)
vwxyz: means within a column lacking a common superscript letter differ ($p<0.05$)