THE EFFECT OF ACID MARINADES ON *LISTERIA MONOCYTOGENES*, SHELF-

LIFE, MEAT QUALITY, AND CONSUMER ACCEPTABILITY OF BEEF

FRANKFURTERS

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Jordan Whitney James Bowers

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DISSERTATION ABSTRACT

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Jordan Whitney James Bowers

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The USDA-FSIS requires processors producing ready-to-eat (RTE) meat and

poultry products exposed to the environment after the primary lethality step to employ

alternatives to kill or inhibit the growth of *Listeria monocytogenes* (LM). Acid

marinades are in use as LM inhibitors, pH controllers, humectants and flavor enhancers.

However, these acid marinades have the potential to negatively affect product quality and

consumer acceptability. Acid marinades: (i) control (no marinade addition) (C); (ii)

sodium lactate (2%; wt/wt) (SL); (iii) potassium lactate (2%; wt/wt) (PL); (iv) sodium

citrate (0.75%; wt/wt) (SC); and (v) sodium lactate (2%; wt/wt)/sodium diacetate (0.25%;

wt/wt) (SLSD) were incorporated into beef frankfurters. Untrained sensory panels

evaluated treatments (8-point Hedonic scale) weekly for 12 weeks. Beef frankfurters

were inoculated (10^7 log CFU/mL) with a streptomycin-resistant (1500µg/mL) strain of

LM known to be pathogenic to humans and stored at 4°C. Samples were enumerated

weekly for, aerobic plate count, psychrotrophs, and LM. SL, PL, and SC treatments did

not adversely affect consumer acceptability through 12 weeks although, SL/SD treatment

was significantly ($P \leq 0.05$) less preferential across all sensory attributes. SL/SD

treatment negatively affected product quality, but was able to control APC, PSY, and LM

levels. SC performed similarly to the control throughout the 8, 9, and 10 week storage

periods, providing no benefit for inhibiting LM or extending shelf-life of the beef

frankfurters. In conclusion, 2% SL, 2% PL, and 2% SL/0.25% SD may be effective LM

inhibitors, but changes in SL/SD treatment formulation should be studied to increase

product quality.

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INTRODUCTION

According to the Centers for Disease Control and Prevention (2009), *Listeria monocytogenes* (LM) is responsible for approximately 2,500 illnesses and 500 deaths, and is the leading cause of pathogen-related food borne recalls in the United States. Ready-to-eat (RTE) foods, like frankfurters, are the foods implicated in *Listeria* recalls/outbreaks, as they are uniquely different from other foods that must be cooked before they are consumed (Schwartz *et al*, 1988; Barnes *et al*, 1989; CDC, 2000; Gottlieb *et al*, 2006). *Listeria* contamination occurs by post-processing cross-contamination, which is why beyond the initial cook step by the manufacturer, RTE products are not required to go through an additional kill step before consumption. They are intended to have longer shelf-lives than raw products and as such, require additional action to prevent and/or reduce the occurrence of pathogen contamination and proliferation during that extended refrigerated storage periods. Unlike many other food borne bacteria, pathogenic or otherwise, LM is able to grow at the refrigeration temperature of 4°C. Most other bacterial growth is limited or inhibited at this temperature.

The United States Department of Agriculture Food Safety Inspection Service (FSIS) enforces zero-tolerance standards for LM in RTE foods (FSIS, 2003). FSIS and the Food and Drug Administration (FDA) developed a regulatory policy stating that,

"plants producing high-risk, ready-to-eat meat and poultry products must develop scientifically validated *Listeria monocytogenes*-control programs, which are stratified according to the number of control measures taken" (Gottlieb *et al.*, 2006). The policy called the FSIS *Listeria* Interim Final Rule (*Listeria* Rule) was enacted in October 2003. The *Listeria* Rule affects any plant that produces a ready-to-eat meat or poultry product that is exposed to the environment after the primary lethality treatment, cooking. The rule establishes three post-primary-lethality treatments meant to control or eliminate LM contamination. The following are the 3 alternative strategies: Alternative 1 – Employment of <u>both</u> a post-primary-lethality treatment and a growth inhibitor (antimicrobial or process), Alternative 2 – Employment of <u>either</u> a post-primary-lethality treatment or a growth inhibitor Alternative 3 - Employment of a sanitation program to control LM in the plant environment and on the product itself (Gottlieb *et al.*, 2006). The food processing facility's hazard analysis and critical control point (HACCP) plan must include the selected alternative utilized in the facility. Based on these required alternatives, further processing facilities have begun to utilize acid marinades in the raw products including, sodium lactate, potassium lactate, sodium citrate and sodium diacetate (Brewer *et al.* 1991). Acid marinades are in use as *Listeria* growth inhibitors, pH controllers, humectants and flavor enhancers (Brewer *et al.*, 1991; Gottlieb *et al.*, 2006) and are incorporated into the raw product and/or post-processing steps.

Sodium lactate (SL) is an organic acid that is used in RTE meat products as a *Listeria* inhibitor and shelf-life extender and is sometimes used as a replacement, at least partially, for sodium chloride (NaCl) resulting in a less-salty taste (Angersbach, 1971;

Houtsma *et al*., 1994 and 1996). SL is permissible at a level up to 3% in a concentrated

form (USDA-FSIS, 2000; Purac America, 2008). Weaver and Shelef (1993) showed that

3% sodium lactate SL inhibited LM growth from an inoculated level of 4 to 5 log CFU/g

on pork liver sausages up to 50 days at 5°C. Additionally, 2.5% SL with 0.25% sodium

acetate inhibited LM (3 log CFU/g) after heating on sliced, vacuum packaged servelat

sausage stored at 4 and 9°C (Blom *et al*., 1997). Bedie *et al*. (2001) reported that LM

inoculation of 3 to 4 log CFU/cm^3 on peeled vacuum packaged pork frankfurters yielded

inhibition of the LM with 3% SL addition for a broad range of 20 to 70 days while

complete inhibition for 90 days when doubled to 6%. SL can be an effective inhibitor of

LM alone, but synergistic antimicrobial properties can be achieved when SL is combined

with other organic acids and/or their salts. In bologna, when 2% SL was combined with

0.5% sodium acetate and 1% sodium bicarbonate their effect was less than when they

were added singly (Wederquist *et al*., 1994 and 1995). This was attributable to the

increase in pH above 7.0 caused by the addition of sodium bicarbonate.

Sodium Diacetate (SD) is a generally regarded as safe (GRAS) chemical and is

derived from acetic acid and the sodium salt of acetic acid (Code of Federal Regulations,

title 21, section 184.1754; Jarchem Industries Inc, 2003). It is available in a white

crystalline powder that is characterized by a vinegar odor that in higher concentrations

can affect the organoleptic qualities of the food (Jarchem Industries Inc, 2003). SD

combined with SL has been shown to exhibit anti-listerial activity in turkey slurries

(Schlyter *et al*., 1993). Though SD is a weak acid and it, theoretically, should not affect

product pH significantly. Islam *et al*. (2002) demonstrated that in turkey frankfurters

dipped in 20% wt/vol solution (0.25% product conc.) SD significantly ($P < 0.05$) lowered

the frank surface pH. Untreated franks had a surface pH of 5.75 and SD addition lowered

the pH to 4.58 to 4.69 (Islam *et al*., 2002). In this same study a 25% wt/vol SD solution

reduced LM levels at 4°C for 10 days when aerobically packaged (Islam *et al*., 2002).

They also found that the inhibitory effect of SD was increased with decreasing storage

temperature (Islam *et al*., 2002; Ahmad and Marth, 1989).

       Potassium lactate (PL) has many of the same uses as SL in RTE foods, but it is

sometimes considered a less-preferential choice as compared to SL because of its a bitter

flavor (Weaver and Shelef, 1992). According to de Vegt (1999), PL decreases the

oxidation of meats during refrigeration, increases meat tenderness, color, and flavor

stability, in part, by increasing water-holding capacity (hygroscopicity). Porto *et al*.

(2001) found that when PL (2% and 3%) was added to the formulation of mixed-species

frankfurters, it was effective at inhibiting the growth of LM (~1.6 log10 CFU and ~1.4

log 10 CFU, respectively) per package at 4°C for up to 90 days. Weaver and Shelef

(1992) reported that PL was listeriostatic when applied at a concentration of 3% wt/wt to

pork liver sausages at 5°C for 50 days.

       Sodium citrate (SC) is another buffered organic acid that is used to control pH, act

to bind water (lower water activity), enhance flavor, and inhibit growth of food borne

pathogens in foods such as, ice cream, candy, gelatin desserts, jams, and RTE meats

(http://www.cspinet.org/reports/chemcuisine.htm; de Vegt, 1999). A study conducted at

a Dutch university determined the minimum concentrations of SL and SC needed to

inhibit the growth of LM (de Vegt, 1999). Addition of 2.5% SL was needed to

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completely inhibit the growth of LM in this study, whereas, 7.0% SC was needed for the same effect. The same study also determined that 6 times as much SC was needed to fully inhibit *Salmonella* and *E. coli* O157:H7, as was needed when utilizing SL. Palumbo *et al*. (1994) determined that when acetic acid and citric acid (both at 2.5%) were combined and applied to frankfurters as a secondary lethality step, inoculated LM was inhibited up to 90 days at 5°C.

The objective of this study was to validate the currently used organic acid marinade's effect at controlling LM on beef frankfurters stored at 4°C. These marinades have the potential to negatively affect product quality and consumer acceptability. There has been little research on the acid marinade's effect on meat quality, shelf-life, and consumer acceptability and as a result, this was also studied. This research is important to the further processing meat industries for the purpose of validating current use, functionality, and usefulness in inhibiting the growth of *Listeria* and spoilage microorganisms.

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CHAPTER II

LITERATURE REVIEW

Cause for Concern: *L. monocytogenes* in RTE Foods

According to the Centers for Disease Control (2008), *Listeria monocytogenes* (LM) is responsible for approximately 2,500 illnesses and 500 deaths annually, and is the number one cause of pathogen-related food borne recalls in the United States. *Listeria monocytogenes* entry into ready-to-eat (RTE) foods occurs by post-process cross-contamination, specifically in franks during stripping, packaging, handling, and in deli loaves during casing removal, slicing and packaging. Therefore, in most of the cases of contaminated meat products, LM is primarily found on the surface of the product (Farber and Peterkin, 1991). Additionally, there has been a positive correlation between contaminated processing surfaces and plant environments with the presence of *Listeria monocytogenes* on finished RTE products (Chasseignaux *et al*., 2001; Heir *et al*., 2004; Berzins *et al*., 2006). Definitive linking of *L. monocytogenes* to specific foods is difficult as the incubation period can be days to weeks in some cases (Low and Donachie, 1997). Because *L. monocytogenes* is so adept at growth during refrigerated storage, the simple convenience of ready-to-eat foods and their extended shelf-life can lend itself to an increased incidence of food borne listeriosis

<u>*Listeria monocytogenes*</u> Characteristics

Listeria monocytogenes is the bacteria responsible for the illness known as listeriosis. Eighty-two years ago LM, then called *Bacterium monocytogenes*, was recognized to cause listeriosis in guinea pigs and rabbits (Murray *et al*., 1926). *Monocytogenes* was chosen as the species name because of a monocytosis found in the infected animals (Murray *et al*., 1926). A few years later (1929), Nyfeldt was the first to isolate the bacterium from an infected human and in 1940, and Pirie was responsible for the bacterium's current name, *Listeria monocytogenes* (Gray and Killinger, 1966).

Currently within the genus *Listeria*, there are six species. *L. monocytogenes* is the species most often implicated with human listeriosis and isolated from infected individuals and contaminated ready-to-eat foods (Allerberger, 2002). The other five species in the genus are *L. grayi*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. innocua* being the most frequently occurring non-pathogenic strain of Listeria (Allerberger, 2002; Farber and Peterkin, 1991). With the exception of *L. innocua* and *L. monocytogenes*, the other species of *Listeria* infect animals (Farber and Peterkin, 1991). There are 13 serovars of *L. monocytogenes* that are known to be pathogenic to humans, and of these, 1/2a, 1/2b and 4b are the most common serovars implicated with listeriosis in humans (Farber and Peterkin, 1991). The causative serovar of listeriosis worldwide is 4b; however, among cases involving meat products, serovar 1 is most predominant (Farber and Peterkin, 1991).

Listeria monocytogenes is a Gram-positive, non-sporeforming, facultative anaerobic rod, meaning that it can grow with or without the presence of oxygen, though

growth is inhibited in complete absence of oxygen (Gray and Killinger, 1966). This facultative characteristic was noted when LM was shown to grow well on both vacuum packaged and aerobically packaged chicken breast and beef samples (Carpenter and Harrison, 1989; Dickson, 1990). LM grows through a wide range of temperatures, 1 to 45°C; optimally between 30 and 37°C (Juntilla *et al*., 1988; Walker *et al*., 1987). *Listeria* exhibits a tumbling motility at room temperature (25°C) as a result of peritrichous flagella. Motility is scant when incubated at 37°C (Peel *et al*., 1988).

<u>Persistence in the Processing Environment</u>

Since *Listeria* contamination occurs by post-process cross-contamination, it is intuitive to think that it's persistence in the processing environment would play a major role in product contamination, pre-packaging. In processing plants, LM is found in areas that may not be easily reached with sanitation procedures, especially those that are damp or moist, such as floor drains, undersides of equipment and air handling systems (CDC, 2008). Processing environments are inherently wet areas, because of the liberal use of water in the plant during regular operation and during sanitation and can persist in the environment for long periods of time. Studies have shown results indicating that it may be unrealistic to think that *Listeria* spp. can be completely removed from processing environments, but they have noted that proper cleaning and sanitizing, as well as, good manufacturing practices (GMPs) can result in effective control of the pathogen (Tompkin *et al*., 1999). Processing equipment can be very complex and should be able to be dismantled for easy cleaning, but this is not always the case. Conveyor belts are especially problematic as they can be very difficult to clean and contain many niches

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where *Listeria* can harbor. Cleaning of these types of surfaces can be tedious and include harsh alkali and acidic solutions, steam and hot water, ultrasonic treatments (Tolvanen *et al.*, 2007, 2009).   Hudson and Mead (1989) found that *Listeria* contaminated surfaces and equipment were responsible for *Listeria monocytogenes* found on 50% of post-processed oven-ready chicken carcasses in a processing plant. They found no *Listeria* species on freshly slaughtered carcass neck skin sections.   The authors consistently found LM on evisceration equipment, the conveyor to the packaging area, evisceration-line drain water and the neck-skin trimmer. Additionally, Keeratipibul and Lekroengsin (2008) determined that ready-to-eat chicken meat products were contaminated by *Listeria* spp. contaminated direct-contact surfaces, especially, conveyor surfaces in direct contact with the product. They also determined that, the initial prevalence of *Listeria*, length of production time, concentration and length of sanitizer contact time were highly correlated (R^2=0.874) with *Listeria* spp. prevalence on direct contact surfaces. Based on these results, the authors recommended thorough pre-production cleaning and sanitizing, as well as thorough cleaning whenever possible during production, *i.e.*: breaks.

In addition to food processing equipment surfaces as sources of contamination for RTE foods, hands of food workers have been shown to harbor *L. monocytogenes*. Kerr *et al.*, 1993, studied the prevalence of *Listeria* spp. on the hands of food workers and non-food workers and found the hands of food workers to be significantly ($P < 0.015$) more likely to harbor *Listeria* spp. than non-food workers' hands. They found *L. monocytogenes* on the hands of retail bakers, fishmongers, grocers, meat product manufacturers, and delicatessen workers. Snelling *et al.*, 1991, studied the survival of *L.*

monocytogenes inoculated onto fingertips after being suspended in either saline or milk and determined that milk had a protective effect for LM, which may help explain why cheeses and milk have been implicated in previous recalls associated with *L. monocytogenes*. Genigeorgis *et al.*, 1990, examined *Listeria* prevalence on the hands and gloves of workers in a turkey processing plant and found 10% of workers were contaminated with *Listeria* that were involved with chilled, raw turkey, and 16.7% of workers handling and packaging turkey cuts were contaminated. Furthermore, 30% of the workers tested harbored *Listeria* spp. on their hands and gloves. As the turkey carcasses and parts progressed through the plant (hanging to cutting to packaging) *Listeria* incidence increased from 16.7, 33.3, and 40%, respectively. Clearly, the presence of *Listeria* spp. on food contact surfaces, processing environments, and the hands of individuals in contact with RTE foods is a major food safety concern and steps must be taken to control the presence and/or growth of the organism whenever possible.

<div align="center">Outbreaks</div>

In the past 3 decades, there have been a number of confirmed food-borne outbreaks of listeriosis caused by *Listeria monocytogenes*. A Canadian outbreak of type 4b *L. monocytogenes* occurred in 1981 involving coleslaw. Of the 41 cases reported, 24 involved pregnant women which resulted in a 27% mortality of the fetus or child and an overall adult mortality rate of 28.6% (Schlech *et al.*, 1983). The contamination was deemed to be caused by cabbage fertilized by manure from a sheep farm in which two animals from the flock died of listeriosis. Post-harvest, the cabbage was stored in a cold-

storage shed. This essentially served as a selective enrichment for the *L. monocytogenes* based upon its psychrotrophic nature (Schlech *et al*., 1983).

In 1983, an outbreak involving properly pasteurized milk occurred in Massachusetts resulting from a 4b strain of LM. In this case, 29% mortality was recorded. The cause of contamination was found to be dairy cattle that were known to be affected by listeriosis at the same time as the outbreak occurred (Fleming *et al*., 1985). This particular outbreak was the first of its kind to suggest a possible heat resistance of *L. monocytogenes*.

A predominant outbreak involving Mexican-style soft cheese occurred in California in the first 8 months of 1985 brought listeriosis, specifically 4b *Listeria monocytogenes* to the forefront of concern for regulatory agencies and food manufacturers. This outbreak caused 142 cases, including 49 adult, out of which 48 were either elderly or immunosuppressed. Forty-eight deaths were reported (30 fetuses or newborns and 18 non-pregnant adults, accounting for a 34% mortality rate (Linnan *et al*., 1988). None of the farms supplying milk to the cheese manufacturer were found to be contaminated, though samples from the manufacturer did test positive for LM. As a result of this outbreak, the implicated plant was closed and the affected cheese was recalled. The 1985 Mexican-style cheese outbreak was the first recorded of its kind in which a specific food was identified and recalled during the actual outbreak (Farber and Peterkin, 1991).

An outbreak spanning from 1983 to 1987 in Switzerland involved soft cheese and accounted for 122 cases and 31 deaths; serotype 4b of LM was involved (Bille, 1990).

The above cases involved a specific food, but there have also been cases in which epidemiology linked food(s) to an outbreak. As such, the first case to link fish or fish products to a listeriosis outbreak occurred in New Zealand in 1980 (Lennon *et al.*, 1984). As with Salmonellosis, antacids were proposed to increase the incidence of listeriosis in a raw vegetable outbreak in Boston that involved 4b LM and caused gastrointestinal distress (Ho *et al.*, 1986).

The first well-documented case involving a meat product was one with RTE frankfurters. Turkey frankfurters were determined to be the cause of a case when a woman, immunocomprimised by cancer treatment, heated a turkey frank for ~1 minute in the microwave and became ill. Because of the possibility that microwaving may not be sufficient to inactivate the bacterium, 600,000 lbs of the product was recalled (Barnes *et al.*, 1989). Of 154 listeriosis cases from a study spanning 1986 to 1987 in the United States, 20% of the sporadic cases were linked to either undercooked chicken meat or uncooked RTE hotdogs (Schwartz *et al.*, 1988).

In another case in May of 2000, an outbreak of listeriosis was linked to contaminated turkey deli meat. The outbreak was responsible for 29 cases of illness in 10 states which resulted in 4 deaths and 3 stillbirths. The company, Cargill Turkey Products, Inc., was implicated in December of that year and subsequent production and distribution of their RTE foods was halted (CDC, 2000).

Arguably, one of the largest (27.4 million lbs fresh and frozen recalled) and most significant recalls of ready-to-eat foods occurred in 2002 in a multistate outbreak of turkey delicatessen meat from a Pilgrim's Pride plant in Pennsylvania (CDC, 2002).

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Fifty-four cases were reported, 8 of which were fatalities and 3 stillbirths all resulting from a single strain of *L. monocytogenes* 4b (Gottlieb *et al*., 2006). Eight patients were pregnant women, 4 were neonates, and 30 patients were considered immunocomprimised because of varying illnesses or treatments (Gottlieb *et al*., 2006). Stemming from this outbreak, the USDA and FSIS released new regulations governing the production and testing of ready-to-eat meat and poultry products (FSIS, 2003 and 2005).

Ubiquity

LM is a ubiquitous organism that is found in soil, silage, sewage, vegetables, surface water samples, human and animal feces, sheep, goats, cattle, soft-ripened cheeses like Camembert and Brie, pasteurized and unpasteurized milk, raw and cooked meat, poultry and fish (Weis and Seeliger, 1975; CDC, 2008). It has been estimated from fecal samples that 5 to 10% of the general population of humans are healthy carriers of *Listeria monocytogenes* and they may shed the organism for long periods of time (Farber and Peterkin, 1991). Additionally, animal carriers of *Listeria* spp. range from 1 to 5%; though, with both humans and animals, these rates may be higher depending upon the methods of detection (FDA/CFSAN, 2003; Farber and Peterkin, 1991). Fecal presence of LM in individuals and animals does not necessarily indicate Listeriosis infection, since these rates are based upon healthy carriers (Farber and Peterkin, 1991).

Growth Characteristics

Unlike many other food borne bacteria, pathogenic or otherwise, *Listeria monocytogenes* is able to grow at the refrigeration temperature of 4°C. Most other bacterial growth is limited or inhibited at this temperature, which is why refrigeration is

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generally the first line of defense for maintaining product safety. Freezing of foodstuffs

contaminated with LM is still not sufficient to kill this bacterium (USDA, 1992).

Listeria is also resistant to salt, nitrite and low pH (Farber and Peterkin, 1991).

LM displays growth from pH 4.5 to 9.6, optimally at 7.0 (Farber and Peterkin, 1991).

Parish and Higgins (1989) reported no LM growth at or below pH 4.0, but Choi *et al*.

(1988) observed that LM survived up to 30 days in yogurt at a pH of 4.0 and speculated

that LM may be hardier than coliforms present in the manufacture of milk and cheese

products. Water activity (a_w) is an important factor when considering the survivability of

an organism. Most bacteria cannot survive below an a_w of 0.91, whereas yeasts and

molds are able to (www.foodtechsource.com). As such, LM is 2nd only to *S. aureus* in its

ability to grow at an a_w of 0.920 and survive. In one study, it was reported to grow on

hard salami at a range of 0.79 to 0.86 (Johnson *et al*., 1988). In addition to *Listeria*'s

ability to resist temperature, water activity and pH extremes, it is also resistant to salt, up

to 10%, and nitrite which are both ingredients in cured meat products (USDA, 1992).

Alone, salt and nitrite may not be effective inhibitors of *Listeria*, but synergistically with

refrigeration, they may be more so against it (USDA, 1992; Doyle, 1999; Duche *et al*.,

2002).

The thermal resistance of *L. monocytogenes* has been documented due to

outbreaks involving pasteurized milk and cooked meat products. In milk, Bunning *et al*.

(1988) determined that the high-temperature, short-time (HTST) pasteurization process

(71.1°C for 15 s) was not a sufficient *D*-process to eliminate LM. Some speculation has

arisen, stating that LM's heat resistance may be due to a phenomenon called heat shock.

Basically, if a product is cooked at lower temperature for a period of time before a final higher temperature is established, then the bacteria become tolerant of heat (Farber and Brown, 1990). Based upon this information one could hypothesize that LM could become thermotolerant in ready-to-eat (RTE) refrigerated foods that are temperature abused. Interestingly, research has shown that curing salts may actually increase the thermotolerance of LM in cured RTE meats such as frankfurters and sausages (Farber *et al.*, 1989; Mackey *et al.*, 1990). Zaika *et al.* (1990) determined that heating of frankfurters to an internal temperature of 71°C was sufficient to produce nearly a 3*D* reduction in *L. monocytogenes*. Also, research by Gaze *et al.* (1989) stated that heating of raw meat to 70°C and holding for 2 min was sufficient to kill all *L. monocytogenes* present. This information, though useful, does not necessarily help the consumer, who is ultimately responsible for the preparation and consumption of the RTE meat products. A 3*D* reduction in LM is significant, but it still may not be enough to completely eliminate *L. monocytogenes* on the product, which is why other inhibitory steps must be employed to reduce the incidence of this bacterium in the final product.

Temperature and pH

Generally, low temperatures are effective at keeping most pathogenic microorganisms from proliferating, but *Listeria monocytogenes* is not adversely affected by this treatment as it grows at refrigerated temperatures. The rate of growth of LM can be affected by the type of product produced. Glass and Doyle (1989) determined that although storage at 4.4°C did not curb the growth of LM, certain products did hinder or promote growth differently. The authors reported that during 12 weeks of storage

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(vacuum) and an initial inoculation level of ca. 10^5 *L. monocytogenes* cells per product, greater than 4 logs of growth was observed on wieners (0 to 9 wks). They also reported a 10^3 and 10^4 CFU/g increase of LM on bratwurst, ham and bologna up to 4 weeks. Sliced turkey and chicken products yielded a 10^3 to 10^5 CFU/g increase over 4 weeks and no growth was observed on summer sausages (Glass and Doyle, 1989). Glass and Doyle noted that the greatest amounts of growth occurred when the pH of the product was at or above 6.0 and the least at or below pH 5.0. The pH of the summer sausages, wieners, ham, bologna, bratwurst, turkey and chicken products were 4.8, 6.0, 6.4, 6.3, 6.2, 6.4, and 6.5 respectively (Glass and Doyle, 1989).

<div align="center">Metabolism</div>

L. monocytogenes is a β-hemolytic bacteria meaning that it lyses red blood cells of most mammals. LM produces zones of hemolysis on blood agar and generally horse or sheep blood is used for this determination (Farber and Peterkin, 1991). Hemolysis is caused by expression of a β-hemolysin called listeriolysin-O (LLO) that is similar to the *S. aureus* hemolysin in that it lyses by forming large transmembrane pores in sheep erythrocytes; indicated by a positive Christie, Atkins, Munch-Petersen (CAMP) test that displays increased hemolysis in the vicinity of a *S. aureus* streak (Farber and Peterkin, 1991; Parrisius *et al.*, 1986). Like *L. monocytogenes*, two other species of Listeria are hemolytic, *L. ivanovii* and *L. seeligeri* (Farber and Peterkin, 1991; Allerberger, 2002). Hemolysis testing is important in the differentiation between *L. monocytogenes* and *L. innocua*, the most frequently occurring non-pathogenic species of Listeria (as stated previously).

L. monocytogenes produces acid from L-rhamnose and α-methyl-D-mannoside but not from D-xylose or D-mannitol (Schuchat *et al*., 1991). LM also hydrolyzes esculin (Low and Donachie, 1997). Esculin is a compound paired with bile salts in selective and differential agars for determination of *Listeria* species (www.bd.com, 2008). A positive esculin hydrolysis test for *Listeria* will turn the agar medium black. Based upon the metabolic characteristics of *Listeria monocytogenes*, a single biochemical test is not sufficient to differentiate between *Listeria* spp. and a combination of tests must be utilized.

<p align="center">Pathogenesis</p>

Normally the intestinal tract has protective barriers such as a mucin layer and commensal bacteria that out-compete and stave-off infection. *L. monocytogenes* has developed virulence factors that enable it to bypass these barriers. In order for *L. monocytogenes* to cause listeriosis, the bacterium must invade the host tissues by a process called parasite-directed endocytosis (McGee *et al*., 1988). *L. monocytogenes* is not the only pathogenic bacteria to possess the ability to bypass host intestinal defenses as *Salmonella* Typhimurium also has this ability (McGee *et al*., 1998). The directed endocytosis of LM provides protection from further detection and destruction by the body's immune system (Low and Donachie, 1997; McGee *et al*., 1998). *L. monocytogenes* has the capability once endocytosed by macrophages to multiply within the macrophage cytoplasm, but first, it must survive the toxic environment of the phagosome, specifically the superoxide radical (McGee *et al*., 1988). LM does this by producing a chemical called superoxide dismutase (Welch, 1987). LM escapes the

<p align="center">18</p>

phagosome by expression of LLO which lyses the phagosomal membrane, releasing the

bacteria into the more favorable and nutrient-rich cytoplasm (Welch, 1987). LLO is

maximally produced during the lag phase, which makes sense considering it is needed to

escape the phagosome before it can continue into the log phase of growth (Gaillard *et al*.,

1986). Free of the phagosome, *L. monocytogenes* propels itself intracellularly by a

unique process called actin polymerization (Tilney *et al*., 1990). LM produces a polar

tail of actin filaments that pushes it through the cytoplasm to the outer cell membrane

(Tilney *et al*., 1990). This process has been determined to occur 2 hours post infection

(Portnoy *et al*., 1988). Once LM has propelled itself to the cell membrane, it continues

until a philopodium is formed (Vasquez-Boland *et al*., 1992). The philopodium is like an

extension of the cell membrane that then pushes into the membrane of a neighboring cell

creating an indentation (Vasquez-Boland *et al*., 1992). At this point, LM produces a

phospholipase that lyses the cell membrane allowing it to enter the adjacent cell without

ever being exposed extracellularly (Vasquez-Boland *et al*., 1992). This is another way *L.

monocytogenes* avoids humoral immune destruction and spreads throughout the body.

The infective dose is currently unknown, but is dependent upon the strain ingested

and the susceptibility of the person exposed (FDA/CFSAN, 1996; CDC, 2008). Most

healthy individuals who may consume *Listeria monocytogenes* are unaffected by it. This

may be due to host-immunity established by previous subclinical exposures to LM or

other Gram-positive bacteria that share similar antigenic structure with *Listeria* (Munk

and Kaufmann, 1988).

Listeriosis infection is greatly increased by immunocomprimised individuals, specifically pregnant women, organ transplant patients, those with HIV/AIDS, cancer patients, and extremes of age (CDC, 2008; Voetsch *et al.*, 2007). Voetsch *et al.* (2007) studied FoodNet data from 1996 to 2003 and found that 24% of cases of listeriosis in individuals over the age of 50 resulted in death.

<u>Symptoms</u>

According to the CDC (2008), symptoms of listeriosis are characterized by nausea, diarrhea, abdominal cramps, headache, fever, lower-back pain and general malaise. *L. monocytogenes* can cross the blood-brain barrier and infect the central nervous system resulting in encephalitis, meningitis and septicemia in severe cases (USDA, 1992). The onset of gastrointestinal symptoms is unclear, but may be as long as 12 hours post-ingestion (FDA/CFSAN, 1992). More severe forms of listeriosis may occur a few days to weeks post-infection (FDA/CFSAN, 1992).

Pregnant women may exhibit a mild flu-like illness and can result in premature delivery, infection of the fetus either through the placenta or during birth, or late term abortion (third trimester) (Doyle, 2003). Neonates, very young children or adults over the age of 60 are especially susceptible (CDC, 2008; USDA, 1992). Voetsch *et al.* (2007) also determined that 16% of laboratory confirmed, reported cases of listeriosis in the U.S. were pregnancy-associated and of these cases, 44% resulted in fetal death. These startling numbers are why doctors urge pregnant women to avoid luncheon meats and other non-reheat ready-to-eat foods.

Diagnosis of *Listeria monocytogenes* is only possible when it is isolated from blood (septicemia), cerebrospinal fluid (meningitis or encephalitis), or feces (gastrointestinal) (FDA/CFSAN, 1992). Fecal isolation is of limited value considering humans may be healthy natural carriers of the bacterium (FDA/CFSAN, 1992). According to the CDC (2008), a blood test for pregnant women is the most effective way to determine *Listeria monocytogenes* infection.

Detection

Detection of *L. monocytogenes* in foods can be a daunting task considering the possible high bacterial background load. Therefore selective broth and agar mediums have been developed. Though, a technique of incubating a food sample in a nonselective broth at 4°C has been effective at exploiting LM's ability to multiply at refrigeration temperatures (Donnelly, 1988). Blood agars are used to test for hemolysis, because pathogenic strains of *Listeria* lyse red blood cells (Farber and Peterkin, 1991; Low and Donachie, 1997). Most of the typical tests involving broth or agar mediums require a series of tests that may take days to complete. The need for rapid detection of LM has prompted the scientific community to develop alternative methods involving the use of DNA probes and monoclonal antibodies (Peterkin *et al*., 1991; Durham *et al*., 1990). In addition to traditional media-based detection methods, there are commercially available, rapid methods including enzyme-linked immunosorbent (ELISA), colorimetric DNA probe, enzyme-linked immunofluorescence (ELFA), polymerase chain reaction (PCR), fluorescence *in situ* hybridization (FISH), and biochips (Brehm-Stecher and Johnson,

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2007). Each of these methods offers the benefits of simplicity, standardization, cost-effectiveness, and rapid detection.

<div align="center">Treatment</div>

Regarding antibiotic treatment, *Listeria monocytogenes*, if detected in time, is susceptible to ampicillin, azithromycin, penicillin, gentamicin, erythromycin, tetracycline, and chloramphenicol (Todar, 2008; Heger *et al.*, 1997; Hof *et al.*, 1997; Lehnert, 2005). Quinolones, like levofloxacin are generally not given to pregnant women because of possible adverse effects to the fetus and are only moderately effective against LM (Todar, 2008; Heger *et al.*, 1997; Hof *et al.*, 1997; Lehnert, 2005). Broad spectrum antibiotics are given to neonates until LM isolation is confirmed. Generally, the same antibiotics given to adults, except quinolones, are administered to neonates (CDC, 2008).

<div align="center">Regulations and Prevention Programs</div>

<div align="center">*Listeria* Interim Final Rule</div>

Zero-tolerance standards exist for *Listeria monocytogenes* in foods. After the 2002 turkey deli meat outbreak and recall, the US Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) and the Food and Drug Administration (FDA) developed a new regulatory policy stating that, "plants producing high-risk, ready-to-eat meat and poultry products must develop scientifically validated *L. monocytogenes*-control programs, which are stratified according to the number of control measures taken" (Gottlieb *et al.*, 2006). The policy called the FSIS *Listeria* Interim Final Rule (*Listeria* Rule) was enacted in October 2003. The *Listeria* Rule affects any plant that produces a ready-to-eat meat or poultry product that is exposed to the environment after

the primary lethality treatment, cooking. The rule establishes three post-primary-lethality

treatments meant to control or eliminate *L. monocytogenes* contamination. One of the

three strategies is required of the product manufacturer and those manufacturers that

utilize a single sanitation step are especially scrutinized for verification and validation

procedures (USDA-FSIS, 2006).

The following are the 3 alternative strategies:

> Alternative 1 – Employment of <u>both</u> a post-primary-lethality treatment and a
>
> growth inhibitor (antimicrobial or process) to prevent the growth
>
> of *L. monocytogenes* on the product until it's recommended
>
> expiration date
>
> Alternative 2 – Employment of <u>either</u> a post-primary-lethality treatment or a
>
> growth inhibitor to prevent the growth of *L. monocytogenes*
>
> Alternative 3 - Employment of a sanitation program to control *L. monocytogenes*
>
> in the plant environment and on the product itself

If alternative 1 is chosen plants will be scrutinized to confirm the effectiveness of

the post-primary-lethality treatment, and if alternative 2 is enacted, then those plants will

be subject to more frequent validation of effectiveness as compared to plants choosing

alternative 1. The plants employing alternative 3 will endure the most frequent validation

inspections, especially those plants producing RTE delicatessen meats and/or

frankfurters, as these products are deemed most at-risk foods for *L. monocytogenes*

contamination (USDA-FSIS, 2004).

In addition, to assist establishments that produce RTE meat and poultry products in effectively following and applying the *Listeria* Rule guidelines, the USDA-FSIS has posted on its website (www.fsis.usda.gov) a document entitled, Compliance Guidelines to Control *Listeria monocytogenes* in Post-Lethality Exposed Ready-to-Eat Meat and Poultry Products.

<p style="text-align: center"><u>Healthy People 2010 Objective</u></p>

In addition to the *Listeria* Rule inception and implementation, another program was established by the US Department of Health and Human Services (USDHHS) as a means of setting a safe-food goal. It was deemed The Healthy People 2010 national health objective and called for a 50% reduction in listeriosis cases, from 5 cases per 1 million in population in 1997 down to 2.5 cases per 1 million in population by 2010 (USDHHS, 2000). According to a study by Voetsch *et al*. (2007), listeriosis cases decreased by 26% from 4.1 cases per million in population in 1996 to 3.1 cases per million in population in 2003. This decrease was partly attributed to the enactment of the aforementioned programs.

<p style="text-align: center"><u>FoodNet</u></p>

Besides the above programs, an active surveillance program (FoodNet) was started to monitor food safety intervention effectiveness by three agencies, the CDC, USDA-FSIS and the FDA. The main goal of FoodNet is to provide surveillance of food-transmissible laboratory-confirmed bacterial pathogens, which includes *L. monocytogenes* (CDC, USDA-FSIS, FDA, 2008). FoodNet is a nationwide program that is limited in some respects by demographics, availability of healthcare and willingness of

the infected person to seek care (Hardnett *et al*., 2004). Chiefly among these limitations is the fact that only laboratory-confirmed results are recorded, so many other cases based upon the aforementioned limitations are not discovered and recorded. From 1996 to 2003, FoodNet recorded a 24% decrease in listeriosis cases associate with *Listeria monocytogenes* (Voetsch *et al*., 2007).

Sanitation

Environmental Monitoring Programs

Levine et al. (2001) noted that in attempts to curb distribution of LM contaminated RTE products, manufactures hold (in some cases >90%) the product until USDA-FSIS tests are determined to be negative for *L. monocytogenes*. Finally, in 2003 the US FDA/CFSAN and CDC issued an update to the *Listeria* action plan (Listeria Rule) that focused attention on strategies for training, research, education, guidance, surveillance, validation and enforcement (FDA/CFSAN, 2003).

Antimicrobial Controls

Various antimicrobials are in use in RTE meat products such as organic acids and their salts, including sodium and potassium lactate, cetylpyridium chloride, sodium diacetate, and sodium citrate; the ingredients are generally-regarded-as-safe (GRAS) for use in these product by the USDA-FSIS (Islam *et al*., 2002). The FDA is not required to test or approve any chemical considered GRAS, but the USDA-FSIS is, however, required to approve their use and usage levels for RTE meat and poultry products (Islam *et al*., 2002). In 2000, the USDA began allowing the use of sodium and potassium lactate up to a concentration of 4.8% w/w in meat products (USDA, 2000).

Surface Pasteurization

Post-processing surface pasteurization is one such strategy that is performed post-packaging; either with steam or hot water baths and is effective at inactivating and/or inhibiting pathogens. Murphy and Berrang (2002) studied the effect of steam and hot-water post-process pasteurization on fully-cooked vacuum-packaged chicken breast strips on *L. innocua* M1, a heat resistance indicator organism for *L. monocytogenes*. Their work determined that both treatments were equally effective at reducing *L. innocua* M1 levels when the sample packages were exposed to the treatments at 88°C for 10 to 35 s. The turkey company, Cooper Farms, in 2001 implemented a post-processing surface pasteurization system in its RTE turkey plant. It now uses an in-line hot-water bath kept at 96.1°C (205°F) to increase the safety of their products. The surface pasteurization process protects the product from being contaminated during post-cook stripping and packaging. To further address the prevention of pathogen growth, the freshly pasteurized products are quickly chilled to bring the surface temperature to below 30°F (-1.1°C) within 10 minutes. The pasteurization process yields the company a 3 log bacterial reduction (Lipsky, 2002).

Irradiation

Irradiation is also utilized as a post-process lethality step in the production of RTE foods. Irradiation was approved in 1990 as a control for food-borne pathogens by the FDA and two forms of radiation can be used, gamma and electron beam (e-beam). The latter form is easier to control and use, as it can be turned-off when not in use. Gamma irradiation uses a radioactive source that is much more difficult to control and electron

beam uses high energy electrical power to create accelerated electrons. Irradiation is measured in kiloGrays (kGy) and the values that are used usually range from 0 to 2.5 kGy. Irradiation at some levels has been known to cause quality changes in the product irradiated. Negative quality effects is one reason other chemical antimicrobial additives are utilized in combination with a lower dose of irradiation to inactivate pathogens such as *L. monocytogenes*. Arguably the most significant reason that irradiation is not preferred as an antimicrobial treatment is the negative consumer perception about irradiation. Therefore irradiation has not been deemed as viable from a consumer standpoint.

Cetylpyridium Chloride

The active ingredient in some mouthwashes, a quaternary ammonia compound known as cetylpyridium chloride (CPC) (GRAS) is in use in raw poultry and beef products (FDA, USDA-FSIS, 2004). It is effective against a number of food-borne pathogens, including *Salmonella*, *E. coli* O157:H7 and *Listeria monocytogenes*. Although, CPC is used on raw meats, it can also be used on RTE meats like frankfurters. Singh *et al*., 2005 published data indicating that CPC as a 1% surface spray on cooked, pre-packaged frankfurters was responsible for a 1.4 to 1.7 log CFU/g reduction of LM during 42 days of storage. They also reported that CPC reduced aerobic plate counts (APC), yeasts and molds, total coliforms on non-inoculated franks to undetectable limits during that same time period at 0 and 4°C. Pohlman *et al*., 2002 found that 0.5% CPC was effective at reducing total coliforms, *S*. Typhimurium, *E. coli* and APCs in ground beef before grinding. CPC also has the added benefit of increasing the consumer

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acceptability of fresh beef by boosting the redness without adversely affecting other attributes like color (Pohlman *et al.*, 2001; Jimenez-Villarreal *et al.*, 2002).

Trisodium Phosphate

Trisodium phosphate (TSP) is a GRAS chemical approved for use by the USDA (1992) for *Salmonella* spp. reduction on poultry carcasses (Giese, 1993). It is known to be effective against *Salmonella*, *E.coli*, *Pseudomonas*, *Staphylococcus*, *Campylobacter* and *Listeria monocytogenes* without adversely affecting sensory attributes (Hwang and Beuchat, 1995; Colin and Salvat, 1996). Pohlman *et al.* (2001) and Capita *et al.* (2001) determined that 10% TSP was effective against LM on ground beef and poultry carcass skin, respectively. TSP is not intended for long term treatment, as it can create negative environmental issues related to residual phosphates leaching into the environment and its high pH.

Preventative Measures: Organic Acids

Ready-to-eat foods are uniquely different from other foods that must be cooked before they are consumed. They are intended to have longer shelf-lives than raw products and as such, require additional action to prevent and/or reduce the occurrence of pathogen contamination and proliferation during that extended storage period. Beyond the initial cook step by the manufacturer, RTE products are not required to go through an additional kill step before consumption. Therefore, other strategies must be incorporated.

To control *Listeria monocytogenes*, acid marinades are in use as *Listeria* inhibitors, pH controllers, humectants and flavor enhancers (Brewer *et al.*, 1991) and are incorporated into the raw product and/or post-processing steps. Acid marinades function

by intracellular acidification, thereby disrupting normal cellular activity, such as respiration and molecular transport (Lado and Yousef, 2007).

Sodium Lactate

Sodium lactate (SL) is used in RTE meat products as a *Listeria* inhibitor and shelf-life extender and is sometimes used as a replacement, at least partially, for sodium chloride (NaCl) resulting in a less-salty taste (Houtsma *et al*., 1996). Sodium lactate ($CH_3CH(OH)COONa$) is available commercially as a 60% wt/wt solution and is permissible at a level up to 3% in a concentrated form, but 4.8% of a 60% wt/wt commercially available form (USDA-FSIS, 2000; Purac America, 2008). Sodium lactate is known to inhibit the growth of a number of food-borne pathogens, such as *C. botulinum*, *B. cereus*, *S. aureus*, *Salmonella* spp. and especially *L. monocytogenes*, to name a few (Angersbach, 1971; Houtsma *et al*., 1994; Houtsma *et al*., 1996). A number of studies have been published on sodium lactate's affect on thermal resistance of pathogens, but each is variable and dependent upon the pathogen tested, the concentration of sodium lactate, the product substrate (meat type) and level of contamination. Juneja (2003) reported that 4.8% sodium lactate in lean ground beef increased the heat resistance of LM from 60°C to 73.9°C. On the other hand, Harmayani *et al*., 1993 found that LM heat resistance was not affected (compared to control) in ground beef containing 1.8% sodium lactate.

Sodium lactate can be an effective inhibitor of LM alone, but synergistic antimicrobial properties can be achieved when SL is combined with other organic acids and/or their salts. Not only does the acid itself affect the growth of LM, but also other

factors like pH, storage temperature, inhibitor concentration, salt content, and nitrite affect LM growth. However, in bologna, when 2% sodium lactate was combined with 0.5% sodium acetate and 1% sodium bicarbonate their effect was less than when they were added singly (Wederquist *et al*., 1994 and 1995). This was attributable to the increase in pH above 7.0 caused by the addition of sodium bicarbonate. In contrast to this result, when 2% sodium lactate was added to bologna (<100 CFU/g LM) alone it was ineffective against LM at 10°C but for 28 days at 5°C is was effective. This research indicates that temperature increases impact the growth of LM on product. Weaver and Shelef (1993) showed that 3% sodium lactate inhibited LM growth from an inoculated level of 4 to 5 log CFU/g on pork liver sausages up to 50 days at 5°C. Additionally, 2.5% sodium lactate with 0.25% sodium acetate inhibited LM (3 log CFU/g) after heating on sliced, vacuum packaged servelat sausage stored at 4 and 9°C (Blom *et al*., 1997). This study indicates that the reduced oxygen atmosphere of vacuum packaging and storage temperatures as low as 4°C are not enough to control LM; and as a result, organic acids were needed. Bedie *et al*. (2001) reported that LM inoculation of 3 to 4 log CFU/cm^3 on peeled vacuum packaged pork frankfurters yielded inhibition of the LM with 3% sodium lactate addition for a broad range of 20 to 70 days while complete inhibition for 90 days was achieved when concentrations doubled to 6%. They suggested that their study suggest the USDA-FSIS consider revising current regulations to increase the acceptable level; sensory and quality affects would need to be considered.

Based upon these studies it can be seen that not only the mere presence of sodium lactate is involved in its effectiveness against LM, but a number of factors as well.

Potassium Lactate

Potassium lactate (PL) has many of the same uses as sodium lactate in ready-to-eat foods, but it is sometimes considered a less-preferential choice as compared to sodium lactate because of its bitter flavor (Weaver and Shelef, 1992). Like SL, potassium lactate ($CH_3CHOHCOOK$) is commercially available as a 60% wt/wt solution (Purac America, 2008). Shelef and Potluri (1995) reported that potassium lactate addition to foods does not typically reduce the pH, though they did discover that sodium lactate does. Porto *et al*. (2001) found that when potassium lactate (2% and 3%) was added to the formulation of mixed-species frankfurters, it was effective at inhibiting the growth of *L. monocytogenes* (~1.6 log10 CFU and ~1.4 log 10 CFU, respectively) per package at 4°C for up to 90 days. Weaver and Shelef (1992) reported that PL was listeriostatic when applied at a concentration of 3% wt/wt to pork liver sausages at 5°C for 50 days. According to de Vegt (1999), potassium lactate decreases the oxidation of meats during refrigeration, increases meat tenderness, color, and flavor stability, in part, by increasing water-holding capacity (hygroscopicity).

Sodium Citrate

Sodium citrate (SC) is yet another buffered organic acid that is used to control pH, act to bind water (lower water activity), enhance flavor, and inhibit growth of food borne pathogens in foods such as, ice cream, candy, gelatin desserts, jams, and RTE meats (http://www.cspinet.org/reports/chemcuisine.htm; de Vegt, 1999). Sodium citrate ($C_6H_5Na_3O_7.2H_2O$) is sold commercially as a white crystalline powder, and is produced by reacting citric acid with sodium hydroxide (Tate & Lyle, Decatur, IL, 2008).

A study conducted at a Dutch university determined the minimum concentrations of sodium lactate and sodium citrate needed to inhibit the growth of *Listeria monocytogenes* (de Vegt, 1999). Addition of 2.5% sodium lactate was needed to completely inhibit the growth of LM in this study, whereas, 7.0% sodium citrate was needed for the same effect. The same study also determined that 6 times as much sodium citrate was needed to fully inhibit *Salmonella* and *E. coli* O157:H7, as was needed when utilizing sodium lactate. Palumbo *et al*. (1994) determined that when acetic acid and citric acid (both at 2.5%) were combined and applied to frankfurters as a secondary lethality step, inoculated *Listeria monocytogenes* was inhibited up to 90 days at 5°C. Scant literature on studies involving sodium citrate in RTE is available, never-the-less, 0.75% sodium citrate was used in the current study to test it's affect on the growth of Listeria monocytogenes, shelf-life, product quality, and consumer acceptability in beef frankfurters.

Sodium Diacetate

Sodium Diacetate (SD) is also a GRAS chemical (Code of Federal Regulations, title 21, section 184.1754). Sodium diacetate is a chemical derived from acetic acid and sodium salt of acetic acid ($CH_3COONa \cdot CH_3COOH$) (Jarchem Industries Inc, 2003). It is available in a white crystalline powder that is characterized by a vinegar odor that in higher concentrations can affect the organoleptic qualities of the food (Jarchem Industries Inc, 2003). SD combined with SL has been shown to exhibit anti-listerial activity in turkey slurries (Schlyter *et al*., 1993). Though SD is a weak acid and it, theoretically, should not affect product pH significantly. Islam *et al*. (2002) demonstrated that in

turkey frankfurters dipped in 20% wt/vol solution (0.25% product conc.) SD did

significantly ($P < 0.05$) lower the frank surface pH. Untreated franks had a surface pH of

5.75 and SD addition lowered the pH to 4.58 to 4.69 (Islam *et al*., 2002). In this same

study a 25% wt/vol sodium diacetate solution reduced *L. monocytogenes* levels at 4°C for

10 days when aerobically packaged (Islam *et al*., 2002). They also found that the

inhibitory effect of SD was increased with decreasing storage temperature (Islam *et al*.,

2002; Ahmad and Marth, 1989).

      LM control is vital to the production and consumption of RTE meats. LM's

unique growth characteristics, survival/growth at refrigeration temperatures, broad pH

range, high salt, and low water activity tolerance allow LM to survive conditions that are

normally unfavorable to other pathogens. Mandated regulations exist for the addition of

control measures for LM in facilities producing and distributing RTE meats. These

regulations specifically note the use of growth inhibitors like organic acid marinades to

control LM. The inhibitors can be added in the raw product formulation or as post-cook

dips. The objective of this study was to validate the currently used organic acid

marinade's, when added in the raw meat formulation, effect at controlling LM on beef

frankfurters stored at 4°C. These marinades (SL, PL, SC, and SL/SD) have the potential

to negatively affect product quality and consumer acceptability. There has been little

research on the acid marinade's effect on meat quality, shelf-life, and consumer

acceptability and as a result, this was also studied. This research is important to the

further processing meat industries for the purpose of validating current use, functionality,

and usefulness in inhibiting the growth of *Listeria* and spoilage microorganisms.

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THE EFFECT OF ANTILISTERIAL INHIBITORS ON BEEF FRANKFURTER CONSUMER ACCEPTABILITY AND LISTERIA PREVENTION

Introduction

According to the Centers for Disease Control and Prevention (2009), *Listeria monocytogenes* (LM) is responsible for approximately 2,500 illnesses and 500 deaths, and is the leading cause of pathogen-related food borne recalls in the United States. Ready-to-eat (RTE) foods, like frankfurters, are the foods implicated in *Listeria* recalls/outbreaks, as they are uniquely different from other foods that must be cooked before they are consumed (Schwartz *et al*, 1988; Barnes *et al*, 1989; CDC, 2000; Gottlieb *et al*, 2006). *Listeria* contamination occurs by post-processing cross-contamination, which is why beyond the initial cook step by the manufacturer, RTE products are not required to go through an additional kill step before consumption. They are intended to have longer shelf-life than raw products and as such, require additional action to prevent and/or reduce the occurrence of pathogen contamination and proliferation during that extended refrigerated storage periods. Unlike many other food borne bacteria, pathogenic or otherwise, LM is able to grow at the refrigeration temperature of 4°C. Most other bacterial growth is limited or inhibited at this temperature.

The United States Department of Agriculture Food Safety Inspection Service (FSIS) enforces zero-tolerance standards for LM in RTE foods (FSIS, 2003). FSIS and the Food

and Drug Administration (FDA) developed a regulatory policy stating that, "plants producing high-risk, ready-to-eat meat and poultry products must develop scientifically validated *Listeria monocytogenes*-control programs, which are stratified according to the number of control measures taken" (Gottlieb *et al*., 2006). The policy called the FSIS *Listeria* Interim Final Rule (*Listeria* Rule) was enacted in October 2003. The *Listeria* Rule affects any plant that produces a ready-to-eat meat or poultry product that is exposed to the environment after the primary lethality treatment, cooking. The rule establishes three post-primary-lethality treatments meant to control or eliminate LM contamination. The following are the 3 alternative strategies: Alternative 1 – Employment of both a post-primary-lethality treatment and a growth inhibitor (antimicrobial or process), Alternative 2 – Employment of either a post-primary-lethality treatment or a growth inhibitor Alternative 3 - Employment of a sanitation program to control LM in the plant environment and on the product itself. The food processing facility's hazard analysis and critical control point (HACCP) plan must include the selected alternative utilized in the facility. Based on these required alternatives, further processing facilities have begun to utilize acid marinades in the raw products; including, sodium lactate, potassium lactate, sodium citrate and sodium diacetate (Brewer *et al*. 1991). Acid marinades are in use as *Listeria* growth inhibitors, pH controllers, humectants and flavor enhancers (Brewer *et al*., 1991; Gottlieb *et al*., 2006) and are incorporated into the raw product and/or post-processing steps.

Sodium lactate (SL) is an organic acid that is used in RTE meat products as a *Listeria* inhibitor and shelf-life extender and is sometimes used as a replacement, at least

partially, for sodium chloride (NaCl) resulting in a less-salty taste (Angersbach, 1971; Houtsma *et al.*, 1994 and 1996). SL is permissible at a level up to 3% in a concentrated form (USDA-FSIS, 2000; Purac America, 2008). Weaver and Shelef (1993) showed that 3% sodium lactate SL inhibited LM growth from an inoculated level of 4 to 5 log CFU/g on pork liver sausages up to 50 days at 5°C. Additionally, 2.5% SL with 0.25% sodium acetate inhibited LM (3 log CFU/g) after heating on sliced, vacuum packaged servelat sausage stored at 4 and 9°C (Blom *et al.*, 1997). Bedie *et al.* (2001) reported that LM inoculation of 3 to 4 log CFU/cm^3 on peeled vacuum packaged pork frankfurters yielded inhibition of the LM with 3% SL addition for a broad range of 20 to 70 days; complete inhibition for 90 days when doubled to 6%. SL can be an effective inhibitor of LM alone, but synergistic antimicrobial properties can be achieved when SL is combined with other organic acids and/or their salts. In bologna, when 2% SL was combined with 0.5% sodium acetate and 1% sodium bicarbonate their effect was less than when they were added singly (Wederquist *et al.*, 1994 and 1995). This was attributable to the increase in pH above 7.0 caused by the addition of sodium bicarbonate.

Sodium Diacetate (SD) is a generally regarded as safe (GRAS) chemical and is derived from acetic acid and the sodium salt of acetic acid (Code of Federal Regulations, title 21, section 184.1754; Jarchem Industries Inc, 2003). It is available in a white crystalline powder that is characterized by a vinegar odor, that in higher concentrations can affect the organoleptic qualities of the food (Jarchem Industries Inc, 2003). SD combined with SL has been shown to exhibit anti-listerial activity in turkey slurries (Schlyter *et al.*, 1993). Though SD is a weak acid and it, theoretically, should not affect

product pH significantly. Islam *et al*. (2002) demonstrated that in turkey frankfurters dipped in 20% wt/vol solution (0.25% product conc.) SD significantly ($P < 0.05$) lowered the frank surface pH. Untreated franks had a surface pH of 5.75 and SD addition lowered the pH to 4.58 to 4.69 (Islam *et al*., 2002). In this same study a 25% wt/vol SD solution reduced LM levels at 4°C for 10 days when aerobically packaged (Islam *et al*., 2002). They also found that the inhibitory effect of SD was increased with decreasing storage temperature (Islam *et al*., 2002; Ahmad and Marth, 1989).

Potassium lactate (PL) has many of the same uses as SL in RTE foods, but it is sometimes considered a less-preferential choice as compared to SL because of its a bitter flavor (Weaver and Shelef, 1992). According to de Vegt (1999), PL decreases the oxidation of meats during refrigeration, increases meat tenderness, color, and flavor stability, in part, by increasing water-holding capacity (hygroscopicity). Porto *et al*. (2001) found that when PL (2% and 3%) was added to the formulation of mixed-species frankfurters, it was effective at inhibiting the growth of LM (~1.6 log10 CFU and ~1.4 log 10 CFU, respectively) per package at 4°C for up to 90 days. Weaver and Shelef (1992) reported that PL was listeriostatic when applied at a concentration of 3% wt/wt to pork liver sausages at 5°C for 50 days.

Sodium citrate (SC) is another buffered organic acid that is used to control pH, act to bind water (lower water activity), enhance flavor, and inhibit growth of food borne pathogens in foods such as, ice cream, candy, gelatin desserts, jams, and RTE meats (http://www.cspinet.org/reports/chemcuisine.htm; de Vegt, 1999). A study conducted at a Dutch university determined the minimum concentrations of SL and SC needed to

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inhibit the growth of LM (de Vegt, 1999). Addition of 2.5% SL was needed to completely inhibit the growth of LM in this study, whereas, 7.0% SC was needed for the same effect. The same study also determined that 6 times as much SC was needed to fully inhibit *Salmonella* and *E. coli* O157:H7, as was needed when utilizing SL. Palumbo *et al*. (1994) determined that when acetic acid and citric acid (both at 2.5%) were combined and applied to frankfurters as a secondary lethality step, inoculated LM was inhibited up to 90 days at 5°C.

The objective of this study was to validate the currently used organic acid marinade's effect at controlling LM on beef frankfurters stored at 4°C. These marinades have the potential to negatively affect product quality and consumer acceptability. There has been little research on the acid marinade's effect on meat quality, shelf-life, and consumer acceptability and as a result, this was also studied. This research is important to the further processing meat industries for the purpose of validating current use, functionality, and usefulness in inhibiting the growth of *Listeria* and spoilage microorganisms.

<center>Materials and Methods</center>

Preparation and Treatment of Beef Frankfurters (Fig. 7)

The basic frankfurter formulation (no acid marinades included) consisted of beef

trimmings (87.9% wt/wt; ca. 28% fat), ice (10%), seasoning (3.1%; salt, dextrose,

monosodium glutamate, onion and garlic powder, sodium erythorbate, spice extractives,

and tricalcium phosphate), sodium nitrite. All spices and seasonings were obtained from

A.C. Legg, Inc., Calera, AL. Ten formulation batches were prepared (2 reps per

treatment) separately to contain (i) no acid marinades (control); (ii) sodium lactate (SL) at

3.3% of a 60% (wt/wt) commercial product, equivalent to 2% pure SL (Trumark, Linden,

NJ); (iii) potassium lactate (PL) at 3.3% of a 60% (wt/wt) commercial product,

equivalent to 2% pure PL (Trumark, Linden, NJ); (iv) 0.75% sodium citrate (SC) (Tate &

Lyle, Decatur, IL); (v) 2% SL (Trumark) combined with 0.25% sodium diacetate (SD)

(Jungbunzlauer, Inc., Ladenburg, Germany). The meat ingredients were mixed and

ground once through a 3/8 in. die plate. The ground meat was then mixed and ground a

second time through a 3/16 in. die plate (Hollymatic® 3000, Thompson Meat Machinery,

Queensland, Australia). The resulting ground meat was partitioned into 13.6 kg (30 lb)

replicates (rep(s)) (10 reps total); 2 reps per treatment. Each 13.6 kg rep was emulsified

with the non-meat ingredients in a bowl chopper (Model C-35 ST, Smith Equipment Co.,

Clifton, NJ) for ca. 5 min and/or until a temperature probe placed in the batter read 10°C

(50°F). Post-emulsification, individual reps were extruded into 22-mm cellulose casings

(Viscofan USA Inc., Montgomery, AL) using a vacuum stuffer (VEMAG Robot 500,

Reiser, Canton, MA). Reps were equally and randomly allocated to smoke racks and

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smoked in a single truck Koch smokehouse (Model 35003, Koch Equipment LLC, Kansas City, MO). After reaching an internal temperature of 68.9°C (156°F) the franks were showered for 10 min with cool water and stored overnight at 4°C. Frankfurters were stripped manually and vacuum packaged according to treatment and rep. Average frankfurter weight was 56 g. The emulsified reps were kept separate throughout the post-emulsification steps. The bowl chopper and vacuum stuffer were cleaned after each individual batch.

Inoculation and Vacuum-packaging of Frankfurters (Fig. 8)

An inoculum containing a streptomycin sulfate-resistant (1500 µg/mL) strain of *Listeria monocytogenes* (LM) Brie 1 was prepared in brain-heart infusion broth (BHI broth; Acumedia Manufacturers, Inc., Lansing, MI) using aseptic techniques. Frankfurters (36 per rep, 72 per treatment, 360 total frankfurters) were aseptically placed onto a sterile aluminum foil sheet. Each frankfurter was inoculated with 10µL of a 10^9 $\log_{10}$ CFU/frankfurter inoculum of *L. monocytogenes* Brie 1, and evenly distributed the length of the frankfurter with a sterile inoculating loop. The inoculated frankfurters were allowed to stand for 5 min at 25°C to encourage bacterial attachment. After the 5 min attachment period, the inoculated frankfurters (1 per bag) were aseptically placed into a vacuum bags (20.3 by 25.4 cm, 3 mil standard barrier, nylon/PE vacuum pouch, Prime Source® Vacuum Pouches, Koch Supplies, Inc., Kansas City, MO), vacuum packaged (Ultravac® 225 Vacuum Chamber Packaging Machine, Koch Equipment LLC, Kansas City, MO), and stored at 4°C.

For shelf-life determination, frankfurters (4 per bag, 144 per rep, 288 per treatment, 1440 total frankfurters) were vacuum packaged (Ultravac UV2100-C, Koch Supplies, Inc.; Koch Equipment LLC) and stored at 4°C for aerobic plate count (APC) and psychrotroph (PSY) determination.

For sensory evaluation, frankfurters (6 per bag X 4 bags per sampling day X 2 reps X 5 treatments X 12 weeks = 2880 total frankfurters) from each rep were vacuum packaged (Koch Supplies, Inc.) and randomly distributed throughout a walk-in cooler (Thermo-Kool®, Mid-South Industries, Inc., Laurel, MS) at 4°C for the duration of the study (1 to 78 d). Each sensory day, frankfurters (3 per rep, 6 per treatment) were sampled for total aerobic and psychrotroph counts.

Microbiological Analyses

Samples were tested on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 78 for total aerobic populations (APC) (Fig. 9) on standard methods agar (PCA; Acumedia Manufacturers, Inc., Lansing, MI); for psychrotrophs (PSY) (Fig. 9) on PCA agar (Acumedia Manufacturers, Inc.); for *L. monocytogenes* Brie 1 (Fig. 10) on BHI agar (Acumedia Manufacturers, Inc.) supplemented with 1500 μg/mL streptomycin sulfate (Fisher Scientific, Fair Lawn, NJ). For APC and PSY determination (Fig. 9), 3 vacuum bags per batch were acquired. There were 3 samples per replicate, 30 total samples that consisted of 3 frankfurters (per batch) that were aseptically placed into a sterile Whirl-Pak™ Filter bag (15 by 23 cm, 710 mL Whirl-Pak™ Filter bag, Nasco, Fort Atkinson, WI). 50 mL of 1% phosphate buffered saline (PBS 1x powder concentrate; Fisher Scientific, Fair Lawn, NJ) was added and shaken by hand for 1 min. Serial dilutions

were made with 9 mL of PBS and 1 mL of each sample was added to 20 mL of PCA, in

duplicate. After setting, APC plates were incubated for 24 – 48 hours at 37°C, and PSY

plates were incubated for 10 d at 4°C. For sensory APC and PSY determination (Fig. 9),

frankfurter samples (3 samples X 2 reps X 5 treatments = 30 total samples) were

aseptically placed into a sterile Whirl-Pak™ Filter bag with 50 mL of PBS (Fisher

Scientific) and shaken by hand for 1 min. Serial dilutions were made with 9 mL of PBS

and 50 µL of each sample was spiral plated (WASP II automated spiral plater,

Microbiology International, Frederick, MD), in duplicate, onto PCA petri plates. After

setting, APC plates were incubated for 24 – 48 hours at 37°C, and PSY plates were

incubated for 10 d at 4°C. For LM determination (Fig. 10), 3 samples per batch (3

samples X 2 reps X 5 treatments = 30 total frankfurter samples) were acquired and each

frank was aseptically transferred to a Whirl-Pak™ Filter bag with 50 mL of PBS (Fisher

Scientific) and shaken by hand for 1 min. Serial dilutions were made with 9 mL of PBS

and 50 µL of each sample was spiral plated (WASP II automated spiral plater,

Microbiology International, Frederick, MD), in duplicate, onto BHI agar supplemented

with 1500 µg/mL streptomycin sulfate and incubated 24 – 48 hours at 35°C. Bacterial

numbers from all incubated petri plates were enumerated with the QCount® machine and

software (Spiral Biotech, Inc., Norwood, MA) and were reported as $\log_{10}$ CFU per frank.

Physical and Chemical Properties

The pH and temperature (°C) of the raw frankfurter batter was obtained before (3

samples per treatment) and after (3 samples per replicate, 6 samples per treatment) acid

marinade addition and was determined with an Accumet® Excel XL20 pH/conductivity

meter (Fisher Scientific, Fair Lawn, NJ). Springiness of fully-cooked frankfurters (25 samples per batch, 50 samples per treatment) was determined (TPA; cm; TA.XTplus Texture Analyser, Texture Technologies, Scarsdale, NY). Proximate analyses for moisture, fat, and protein of the fully-cooked frankfurters from each batch were determined by methods approved and described by AOAC International. Moisture of the fully-cooked frankfurters (25 samples per rep, 50 samples per treatment) was determined by mincing approximately 2 g of each sample, placing sample in an aluminum dish ≥ 50 mm diameter and drying $16 - 18$ h at $100 - 102°$ C in an air oven. After drying, the samples were cooled in a desiccator and weighed. Loss in weight was reported as moisture content (AOAC 950.46Ba). Fat (crude) of fully-cooked frankfurters (5 samples per replicate, 10 samples per treatment) was determined by weighing a $3 - 4$ g sample into a cellulose thimble containing a small amount of sand. Sample and sand were mixed with a glass rod and dried 1 h in a $125°$ C oven. Thimble was removed from oven and cooled. Sample and sand were loosened with a glass rod and a small amount of cotton was placed in top of thimble. Thimble was transferred to Soxtec extraction unit and fat was extracted from sample with 40 mL of petroleum ether in boiling position for 25 minutes and rinsed for 30 minutes. Extraction cup was dried at $125°$ C for 30 minutes, cooled and weighed to calculated fat percentage of sample (AOAC 991.36). Protein (Kjeldahl) of fully-cooked frankfurters (5 samples per rep, 10 samples per treatment) was determined by a method approved by AOAC International, protein factor of 6.25 (AOAC 991.20.I).

Sensory Evaluation

Sensory analysis was performed to evaluate the effect of the treatments on the consumer acceptability of frankfurters formulated with and without acid marinades. An untrained test panel (n = 60; 30 in the A.M. (rep 1) and 30 in the P.M. (rep 2)) was recruited using E-mail postings and flyers from employees and students at Auburn University to evaluate frankfurters for appearance, flavor, texture, juiciness, and overall acceptability on an 8-point hedonic scale as suggested by the Institute of Food Technologists (IFT, 1981). The recommended number of panelist responses per sample product is listed between 50 and 100 (IFT, 1981). Panelist selection criteria were an age of ≥19 years and a willingness to participate. Frankfurters were boiled to re-heat, cut into ca. 2 cm pieces and placed into capped (PL2 clear plastic souffle′ lids, Solo$_®$ Cup Company, Highland Park, IL) plastic sample cups (59.2 mL B200 plastic souffle′s, Solo$_®$ Cup Company) labeled with a random 3-digit code number and kept warm (ca. 82°C) until service (FlavorView C175-C(1)N Heated Cabinet, Intermetro Industries Corp., Wilkes-Barre, PA). Each panelist was given an Institutional Review Board (IRB) approval letter from Auburn University's Office of Human Subjects Research and an evaluation form for each sample and were asked to score each frankfurter sample based on their degree of liking ("like extremely" to "dislike extremely"). Each of the 5 sensory attributes were scored from the same 8-point hedonic scale that ranged from, (1) Like Extremely; (2) Like Very Much; (3) Like Moderately; (4) Like Slightly; (5) Dislike Slightly; (6) Dislike Moderately; (7) Dislike Very Much; (8) Dislike Extremely

(Appendix C). Room-temperature water and unsalted crackers were provided to cleanse

panelists' palates between samples.

Statistical Analysis

Statistical Analysis of the data was conducted using SAS 9.1 software (SAS

Institute, Cary, N.C.). Comparisons were made using Proc Mixed LS means and

significant differences ($P \leq 0.05$) were identified. The experimental unit for expressing

microbiological data was frankfurter.

Sensory Analysis

Organic acids can impart off-flavors to the products that they are incorporated into. Research involving the marinades used in this study and their effect on sensory attributes of beef frankfurters has not been published to the best of our knowledge. Therefore, sensory panel evaluations were administered to determine the effects, if any, of organic acid addition on the organoleptic properties of the frankfurters. These sensory attributes are important to consumers. If the foods are unpalatable, or not aesthetically pleasing to consumers; they will not purchase them.

Sensory panel evaluation results are listed in Table 1. Panelists were asked to evaluate the effect of the treatments on the consumer acceptability of frankfurters formulated with and without acid marinades for appearance, flavor, texture, juiciness, and overall acceptability on an 8-point hedonic scale. The higher the number rating, the less favorable the sensory score. In week 1, the SL/SD treated frankfurters were evaluated significantly ($P \leq 0.05$) higher than all other treatments and the control, across all sensory attributes, scoring from 5.27 to 6.71. Examination of the SL/SD frankfurters were found to be dry, rubbery and had an acetic acid odor therefore, the SL/SD treated frankfurters were served to panelists during week 1, only. When 3% SL and 0.25% SD was added in combination to the raw formulation of turkey deli loaves, a trained sensory panel found it to intensify the turkey flavor of the product (Carroll *et al*., 2007). Turkey deli loaves are minimally seasoned products that do not have intense flavor profiles, which makes it easier for consumers to detect changes in flavor. On the other hand, frankfurters are

intensely flavored. Even so, the flavor and aroma of the SL/SD treated frankfurters were able to overcome the intense seasonings. SD is derived from acetic acid and therefore has the potential to impart a vinegar-like odor (Jarchem Industries Inc, 2003). Weeks 2 thru 12, panelists were asked to evaluate the control, SL, PL, and SC treatments, only. Weeks 1 through 12, flavor was unaffected by SL, PL, and SC treatment addition. This result may be due to frankfurters naturally having a strong flavor profile because they are heavily seasoned and smoked, which may mask any flavor differences. Aktas and Kaya (2001) found that of 1% and 1.5% lactic and citric acid marinated bovine longissimus dorsi muscles, taste and aroma were acceptable up to 1% of each of lactic and citric acid. Somewhat contrary to this result, Mikel *et al*. (2006) found that 2% lactic and acetic acid treated beef strip loin sensory results were unaffected through 112 d of storage. In the current study, texture and overall acceptability were unaffected by SL, PL, and SC treatment addition through the entire storage period (Table 1). Brewer *et al*. (1991) determined that SL addition (2 and 3%) to fresh pork sausages delayed the development of off-flavors for 7 d at 4°C and did not affect product appearance. In weeks 4 and 12, PL and SL, respectively, were evaluated to be significantly ($P \leq 0.05$) more juicy than the other treatments in that week (Table 1). The reason for this result is not known, though it is not likely caused by a change in pH, as pH was not significantly affected by treatment addition (Figure 3). Had pH played a roll in these results, we would have expected SC, as it did significantly ($P \leq 0.05$) raise the product pH pre- to post-marinade addition from 5.59 to 6.31 to be more juicy (Figures 3). Vieson *et al*. (2007) found that beef steak

treatment with 2.5% SL was more flavorful, tender, and juicy than untreated steaks over

an extended period of time.

% Moisture

A number of proximate analyses were performed on the fully cooked frankfurters,

including percent moisture (Figure 1). Percent moisture is a measure of the amount of

water in a food product and is determined by the amount of water driven-off in a drying

oven over a period of time, usually 18 hours. Determining the moisture content of a food

can be important with regards to yield, product quality and shelf-life. Based on these

results, SL/SD treated frankfurters had significantly higher moisture loss, 53.35%,

compared to the control ($P \leq 0.05$). SL/SD frankfurter moisture results may be explained

by the 'fatting out' that occurred during the cook process (Appendices D-F). It can be

speculated that the SL/SD combination disrupted the emulsification of fat and moisture

by the meat proteins, thereby releasing the fat and moisture which cooked-out of the

franks. The meat protein system was unable to effectively bind water; therefore,

moisture loss was significantly higher than the control frankfurters. When the franks

were removed from the smokehouse, 'fat caps' with pockets of oil were seen on the ends

of the franks in the casing (Appendix D). Additionally, fat was seen running the length

of the exterior of the franks within the casings (Appendix E). Upon stripping, franks

were visibly and tactilely greasy (Appendix F). A means to increase meat protein bind

and water-holding capacity (WHC) would be to add a protein isolates such as soy to the

product formulation. Additionally, as the % moisture loss of the SL treatment alone was

not different from the control, but SL/SD treatment was different from the control (Figure

1), lowering the percentage of SD in the formulation may improve the meat protein stability and improve product quality. SL, PL, and SC had similar moisture loss to the control frankfurters (Figure 1). Organic acids are known to impact the WHC of the product that they are incorporated into by acting as humectants and pH controllers (Brewer *et al*., 1991). A study by Samelis *et al*., 2002 determined the moisture loss of frankfurters containing sodium lactate to be 52.66%, whereas in the current study, percent moisture loss was determined to be 52.36%, slightly lower (Figure 1). Control frankfurter data showed that moisture content for the formulation that we used was just above 51%; therefore, SL addition slightly improved moisture bind (Figure 1).

Springiness

Product springiness was also measured and results are displayed in Figure 2. Springiness is the amount that the frankfurter recovers (cm) after being briefly compressed. Springiness can be associated with the amount of moisture in a food, as well as, the texture of the food. In the current study, springiness appeared to be associated with percent moisture. There was no difference in springiness values except for SL/SD frankfurters which were determined to be significantly ($P < 0.05$) less springy than SL, PL, SC and control frankfurters. This treatment had the highest percent moisture loss, as well. Again, the SL/SD treatment destabilized the emulsification ability of the meat proteins of these frankfurters (fatting-out and more moisture loss than the control), and as a result they were rigid and firm. This fact may partially explain why the springiness value was significantly lower than all the other frankfurters (SL, PL, and SC), treated and untreated (control). The SL, PL, and SC treated frankfurters were not

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different from the control. We hypothesized that treatment addition would not adversely affect product quality as measured by proximate analysis and thusly were pleased to find that springiness was not significantly affected by most treatments, except for SL/SD treated frankfurters.

pH

pH is an important factor from a protein functionality standpoint in a further processed meat product. Post-mortem meat pH decline occurs by the breakdown of muscle glucose to lactic acid during rigor development. During this time, muscle pH can decline from 7.0 to 5.3 – 5.7 nearer to the isoelectric point (IP) (5.1), which is considered the pH at which the proteins are the least functional (Lawrie, 1991). As pH declines, loss of protein functionality, specifically, WHC can have adverse effects on emulsion stability. Buffered organic acids can be used to control pH to alleviate the effects of rapid post-mortem pH decline and increase WHC (de Vegt, 1999).

Post-treatment pH results are displayed in Figure 3. SL and PL treatment pH values were not different than the control. PL and SL/SD treatments had similar post-treatment pH values. SL/SD treated frankfurters had pH values similar to the SC treatment and were different from the control ($P \leq 0.05$). Though SD is a weak acid and it, theoretically, should not affect product pH significantly. Islam *et al*. (2002) demonstrated that in turkey frankfurters dipped in 20% wt/vol solution (0.25% product conc.) SD did significantly ($P < 0.05$) lower frank surface pH. Brewer *et al*. (1991) noted that the addition of 2 and 3% SL did not significantly ($P < 0.05$) change the pH of fresh pork sausages. Muscle proteins have a high buffering capacity against changes in pH and

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weak organic acids are not meant to change product meat pH; therefore, these results were expected.

Aerobic Plate Count

One of the objectives monitored during this study was the effect of organic acid treatment on the sustainability of shelf-life in regard to APC and PSY on non-inoculated frankfurters during storage at 4°C. These two parameters were chosen because they are commonly used to determine spoilage of ready-to-eat meat products. Product was considered spoiled when APC and PSY levels reached 5×10^6 log CFU/frank (Rorvig, 2007). APC levels, in general, increased during storage at 4°C (Figure 4). APC counts were less than 2 logs for all treatments and the control at the beginning of the study (Figure 4). SL, PL, and SL/SD treatments were more effective at controlling APCs than the control and the SC treatment (Figure 4). SL, PL, and SL/SD treatments did not reach spoilage during the entire 10 week shelf-life analysis period however, SC reached spoilage at week 5 and the control at week 6 (Figure 4). At the beginning of shelf-life, week 1 to week 2, SC treated frankfurters' APC counts increased 3.5 Logs from 1.84 to 5.34 Log_{10} CFU/frank; just over 1 Log more than the control during the same time period (Figure 4). During weeks 3, 4, 5, 6, 7, 8, 9, and 10, SC was not different from the control (Figure 4). During the course of researching an explanation for this result it was found that SC may, in fact, promote the growth of spoilage bacteria, satisfying a needed growth metabolite (Ryser and Marth, 2007). Week 2 SC counts were significantly ($P \leq 0.05$) higher than that of SL, PL, and SL/SD APC counts, though, not different from the control (Figure 4). Weeks 3, 4, 5, 6, 7, 8, 9, and 10, organic acids (SL, PL, and SL/SD) reduced

APC counts compared to the control (Figure 4). Similar to this study, Carroll *et al*. (2007) found that SL (3%)/SD (0.25%) combination treatment kept APC levels below spoilage for 70 d on turkey deli loaf slices. Brewer *et al*. (1991) reported that increasing total aerobic plate count (TPC) was inversely proportional to SL concentration. They also showed that an initial TPC level of 10^6 log CFU/g increased to over 10^8 log CFU/g in 10 d of storage at 4°C when SL was added to raw product formulation at 1%. On the other hand, when 2% and 3% SL was added incorporated, TPC levels dropped slightly within the same 10 d storage period and temperature. Storage periods of 21 d were necessary for the 2 and 3% SL treatments to exceed 10^8 log CFU/g of TPCs (Brewer *et al*., 1991). At week 10, when the control frankfurters had reached spoilage and the shelf-life study was terminated, SL, PL, and SL/SD treatment levels were more than 10^2 log CFU/frank less than the SC treatment (Figure 4). Drosinos *et al*. (2006) noted that 2, 3, and 4% SL as well as 2, 3, and 4% SL combined with 0.5% sodium acetate was able to provide an additional 10 d of shelf-life on cured cooked meats. Additionally, Ouattara *et al*. (1997) studied the effect of 0.1 to 1% acetic, lactic and citric acids on spoilage bacteria and found that all were effective at inhibiting the growth of the spoilage bacteria. The order of most inhibition was acetic, lactic, and citric acid (Ouattara *et al*., 1997). SL, PL, and SL/SD were effective at controlling APC levels throughout the course of the 10 week storage period (Figure 4). Though the SL/SD treatment's product quality was destroyed (Table 1), the treatment was still able to control APC levels weeks 1 through 10 (Figure 4). Studies have found that the inhibitory effect of SD was increased with decreasing storage temperature (Islam *et al*., 2002; Ahmad and Marth, 1989). Though

treatment water activity was not measured in this study, it is thought that available water

for microorganism growth was not high enough to sustain APC growth because of the

loss of muscle protein emulsion stability (Figure 4). Had the treatment not fatted-out or

been able to bind water to the extent of the control (Figure 1), the product quality would

have been improved, but the bacteriostatic effect may have been lessened.

Psychrotrophs

PSY are bacteria that exhibit growth between 0°C and 20°C; optimally growing

at 15°C or below and are good indication of refrigerated product spoilage, like that of

APCs, as refrigeration is considered to be 4°C (Morita, 1975). Psychrotrophs were

monitored during the shelf-life portion of the study and the results are recorded in Figure

5. PSY counts were collected for a period of 8 weeks. During 8 weeks of storage, PSY

levels increased for all treatments (SL, PL, SC, and SL/SD) and the control (Figure 5).

Through the entire 8 week storage period, SC treated frankfurter PSY levels were not

different than the control PSY levels (Figure 5). SC frankfurters reached spoilage (6.77 x

10^6 log CFU/frank) at week 5 whereas, the control reached spoilage at week 6 (6.91 x 10^6

log CFU/frank) as shown in Figure 5 as was the result seen in the APC data (Figure 4).

PL and SL/SD treatments did not reach spoilage levels through the entire 8 week time

period (Figure 5). SL reached spoilage at week 8 (6.44 x 10^6 log CFU/frank), when the

study was terminated (Figure 5). As with the APC results (Figure 4), PSY counts of SL,

PL, SC, and SL/SD treatments at week 1 were not different than the control (Figure 5).

At weeks 5 and 6 of storage the SL, PL, and SL/SD treated frankfurter PSY counts were

lower than those of the control and SC treatment (Figure 5) however, this trend was not

maintained through weeks 7 and 8 of storage (Figure 5). SC counts were higher than the

Control in weeks, 1, 3, 4, and 5 (Figure 5). SC treated frankfurters were determined to

have no beneficial effect on extending shelf-life compared to that of the control

frankfurters. APC data also showed that (Figure 4) SL, PL, and SL/SD treatments were

effective at controlling the growth of PSY on the frankfurters compared to the control and

SC treatment (Figure 5). Explanations of PSY results are directly related to that of the

APC results in Figure 4, as they are both indications of product spoilage.

Listeria monocytogenes

The focus of the current study was to determine the affect of different organic

acids formulated into beef frankfurters on *Listeria monocytogenes* when inoculated onto

the surface of the frankfurters, vacuum-sealed and stored at 4°C. Individual frankfurters

were inoculated with ~ 7.0 Log_{10} CFU/mL culture of *L. monocytogenes* Brie 1. *Listeria*

data is shown in Figure 6. SL, PL, and SL/SD treatments were found to be effective at

inhibiting the growth of LM (extending the lag phase), through 9 weeks of storage

compared to the control (Figure 6). Porto *et al*. (2001) found that when potassium lactate

(2% and 3%) was added to the formulation of mixed-species frankfurters, it was effective

at inhibiting the growth of *L. monocytogenes* (~1.6 log10 CFU and ~1.4 log 10 CFU,

respectively) per package at 4°C for up to 90 days. Additionally, Wederquist *et al*.

(1994, 1995) found that when 2% sodium lactate was added to bologna (<100 CFU/g

LM) alone, it was effective against LM at 5°C. Weaver and Shelf (1992, 1993) showed

that 3% sodium lactate inhibited LM growth from an inoculated level of 4 to 5 log CFU/g

on pork liver sausages up to 50 days at 5°C, and they reported that PL was listeriostatic

when applied at a concentration of 3% wt/wt to pork liver sausages at 5°C for 50 days.

Bedie *et al*. (2001) reported that LM inoculation of 3 to 4 log CFU/cm^3 on peeled

vacuum packaged pork frankfurters yielded inhibition of the LM with 3% sodium lactate

addition for a broad range of 20 to 70 days. Another study by Blom *et al*., (1997)

reported that 2.5% sodium lactate with 0.25% sodium acetate inhibited LM (3 log

CFU/g) after heating on sliced, vacuum packaged servelat sausage stored at 4°C. Control

and sodium citrate treated frankfurters were consistently greater than 7 logs of LM for the

entire 9 weeks of storage, ending with 9.86 and 10.25 logs, respectively (Figure 6).

Excluding week 1, the control and SC treatment were significantly ($P \leq 0.05$) greater in

LM counts than SL, PL, and SL/SD treatments, which were similar in their LM counts

(Figure 6). Figure 6 illustrates that SC had no inhibitory effect on LM growth. In fact,

by week 9 of shelf-life LM counts for SC peaked at more than 3 logs higher than the

week 1 of the study (7.7 to 10.2 $\log_{10}$ CFU/frank). Contradictory to these findings,

Palumbo *et al*. (1994) determined that when acetic acid and citric acid (both at 2.5%)

were combined and applied to frankfurters as a secondary lethality step, inoculated LM

was inhibited up to 90 days at 5°C. The results of the Palumbo *et al*. study may have

been due to the high concentration of acetic acid in the formulation (2.5%) counteracting

the growth promotion effect of citric acid (Ryser and Marth, 2007). This explanation is

further backed by the data in Figure 6 showing that a concentration of SD 10 times less

(0.25%) than the Palumbo et al study was sufficient to inhibit LM but, a concentration of

0.75% SC was unable to provide similar results. The inability of SC to inhibit LM may

be explained by a higher than control post-marination pH, 6.31, moving the pH closer to

neutrality (Figure 3). *Listeria* resists low pH and can grow between 4.5 and 9.6; optimally at pH 7.0 (Farber and Peterkin, 1991). Therefore, the pH of the SC treated frankfurters was the nearest to the optimal growth pH of *Listeria monocytogenes* (Figure 3). de Vegt (1999) reported the addition of 7.0% SC was needed to completely inhibit LM. SL, PL, and SL/SD treatments ended below their original inoculum levels, with SL/SD having the lowest LM counts (6.0 $\log_{10}$ CFU/frank) (Figure 6). Based upon the current data, 0.75% SC addition provides no benefit to controlling LM or extending shelf-life of beef frankfurters when stored at 4°C but, SL, PL, and SL/SD treatments were effective.

Conclusion

In conclusion, SL, PL, and SC treatments did not adversely affect consumer acceptability of the frankfurters through 12 weeks of storage at 4°C; although, SL/SD treated frankfurters were perceived to be significantly ($P \leq 0.05$) less preferential across all sensory attributes. Springiness was not affected by SL, PL, and SC treatments, except for SL/SD treated frankfurters, which were significantly ($P \leq 0.05$) less springy than all other treatments and control. APC and PSY data showed that (Figure 4 and 5, respectively) SL, PL, and SL/SD treatments were effective at delaying spoilage compared to the control and SC treatment (Figure 5). Based upon this study, 0.75% SC addition provides no benefit for inhibiting LM or extending shelf-life of beef frankfurters when stored at 4°C. SL, PL, and SL/SD treatments were effective at extending the lag phase of LM inoculated frankfurters for 9 weeks of storage at 4°C. The benefits of inhibiting spoilage (APC and PSY) and LM growth are ineffective if the product quality is such that it is unpalatable for human consumption.

Therefore, further research should be performed on different concentrations of SD that may have less negative quality affects, yet continue the LM, APC, and PSY inhibition. Also, the addition of a protein isolate such as soy may help to increase meat system protein functionality.

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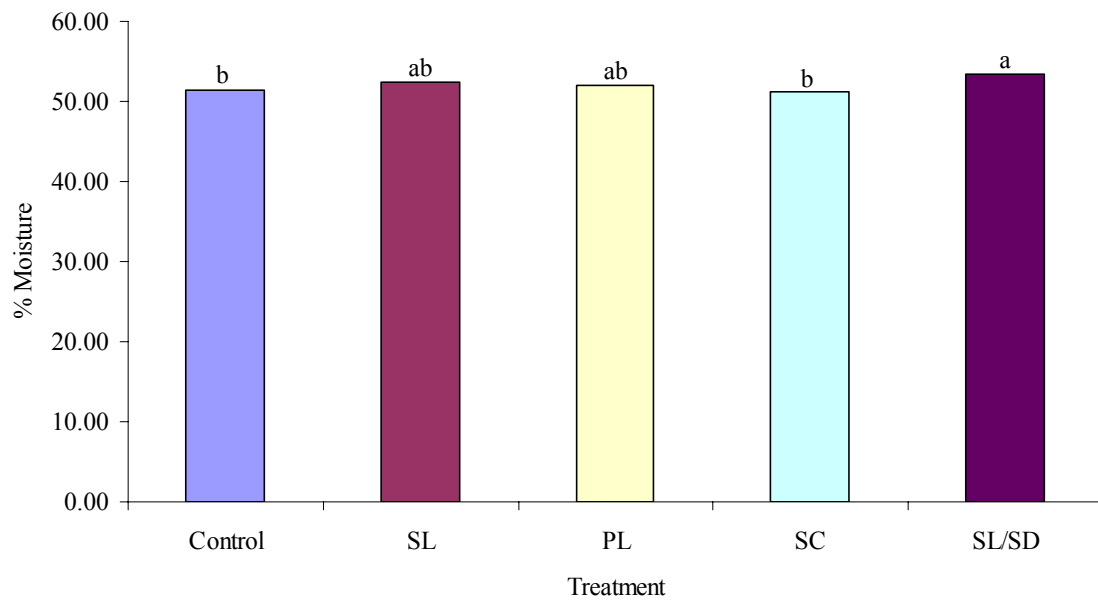
Figure 1. % Moisture results of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%). Standard deviations varied from 0.05 to 0.13.
[a-b]Means with no common superscript letters differ significantly ($P \leq 0.05$)
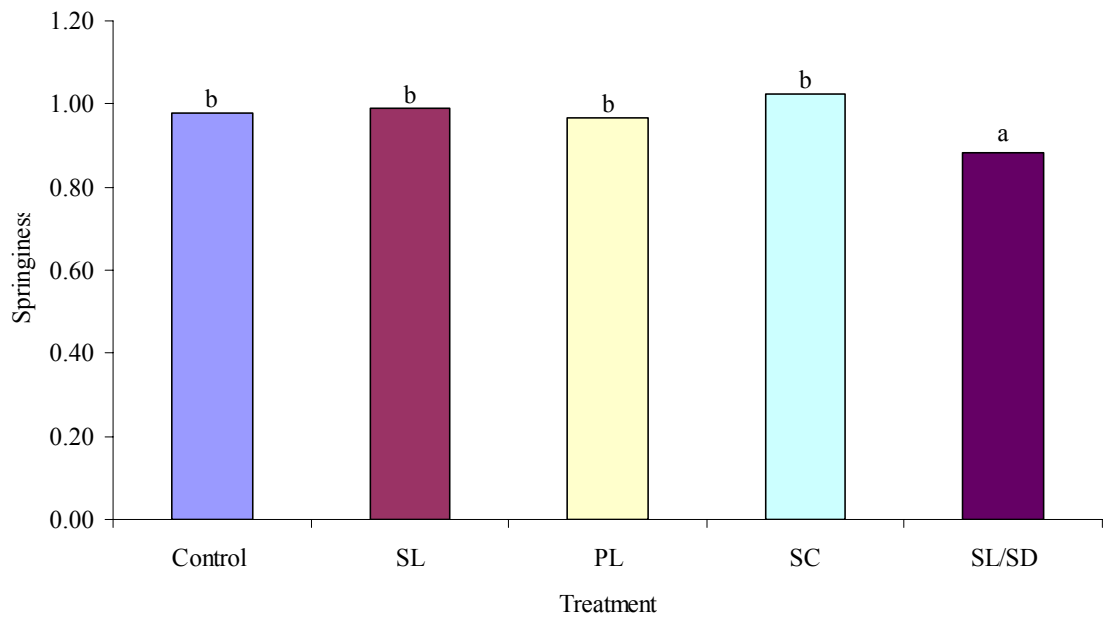
Figure 2. Springiness values of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%). Standard deviations varied from 0.04 to 0.31.
[a-b]Means with no common superscript letters differ significantly ($P \leq 0.05$)
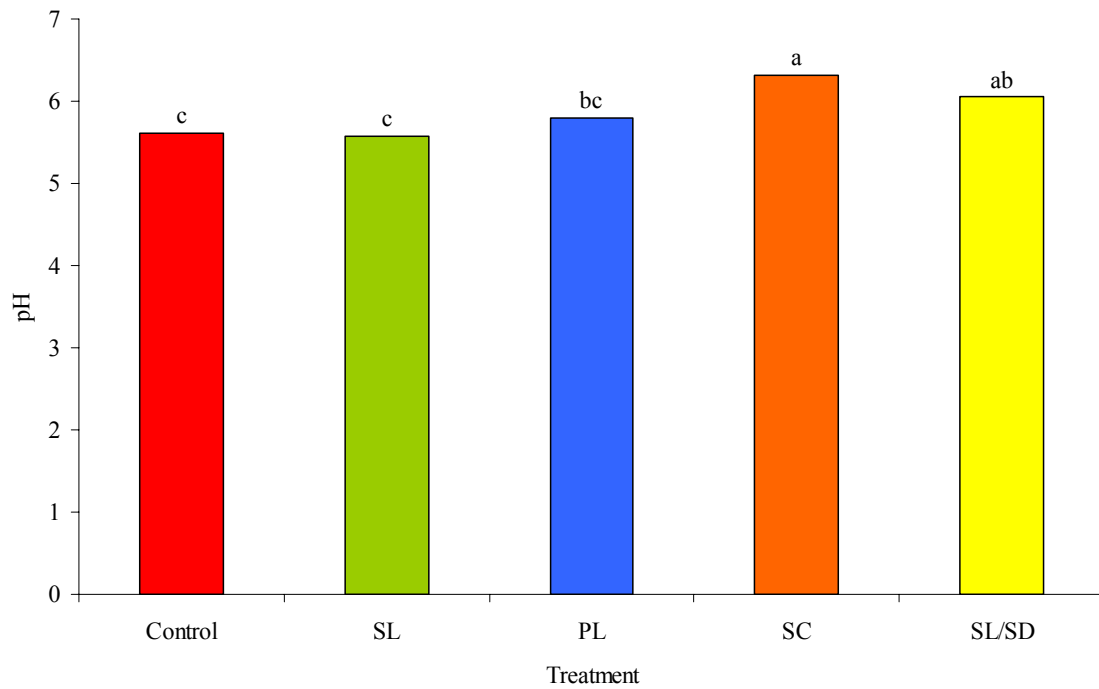
Figure 3. Post-treatment pH values of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%). Standard deviations varied from 0.01 to 0.07.
[a-c]Means with no common superscript letters differ significantly ($P \leq 0.05$)
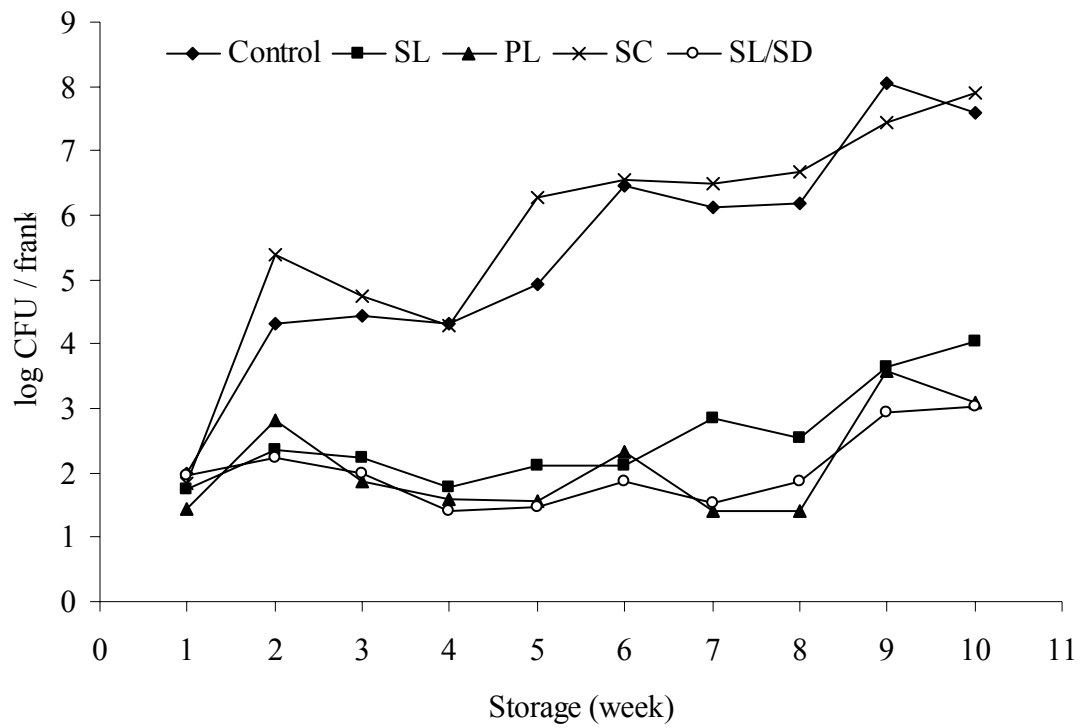
Figure 4. Aerobic plate count (log CFU/frank) of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%) during 10 weeks of storage at 4°C. Standard deviations varied from 0.00 to 2.58.
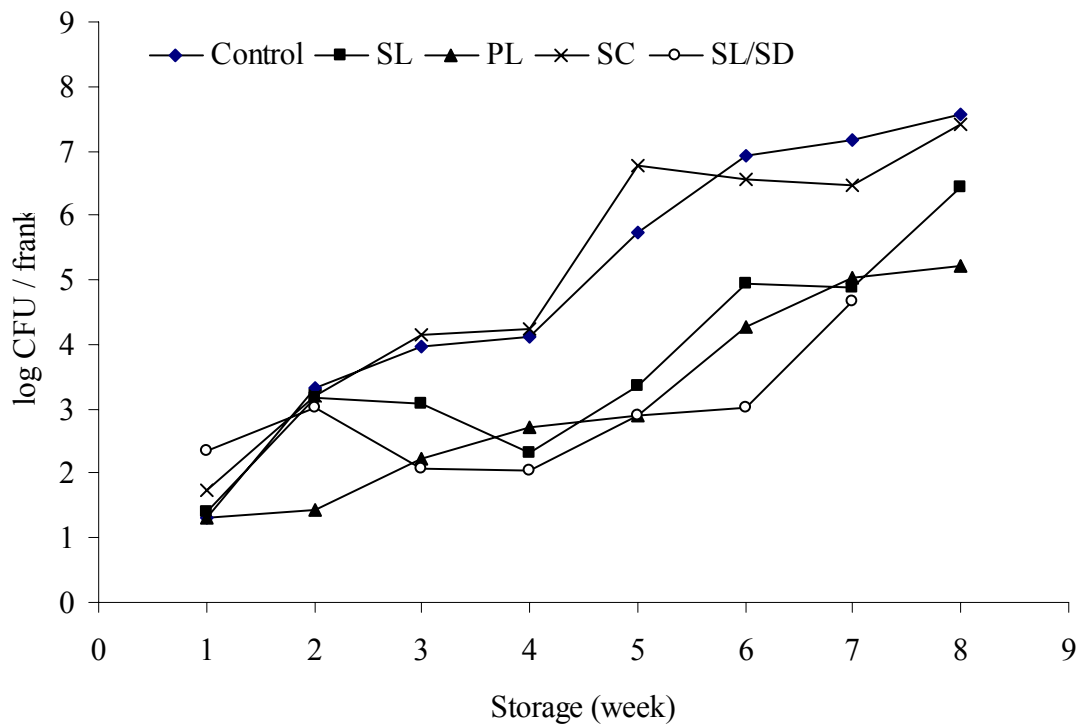
Figure 5. Psychrotroph results (log CFU/frank) of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%) during 8 weeks of storage at 4°C. Standard deviations varied from 0.03 to 1.96.
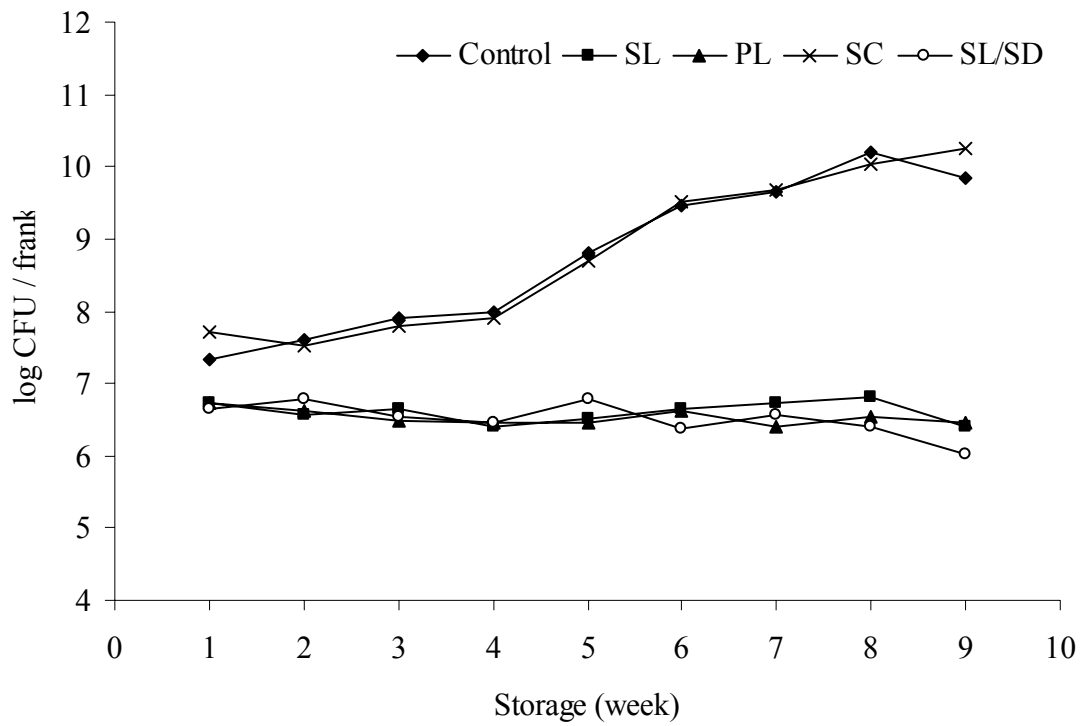
Figure 6. *Listeria monocytogenes* Brie 1 plate count (log CFU/frank) of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%) during 9 weeks of storage at 4°C. Standard deviations varied from 0.00 to 0.47.

Figure 7. Frankfurter Preparation Flowchart Diagram

Beef Trimmings

Mixing / Grinding (3/8" die plate)

Mixing / Grinding (3/16" die plate)

10 Replicates (13.6 kg each)
2 replicates / treatment

Emulsification (Bowl Chopper)
Seasoning, Treatment Addition

Vacuum Stuffing
(22 mm cellulose casing)

Smoking
Internal (68.9° C)

Cooled overnight at 4°C

Stripping and Vacuum Packaging
(According to treatment & rep)
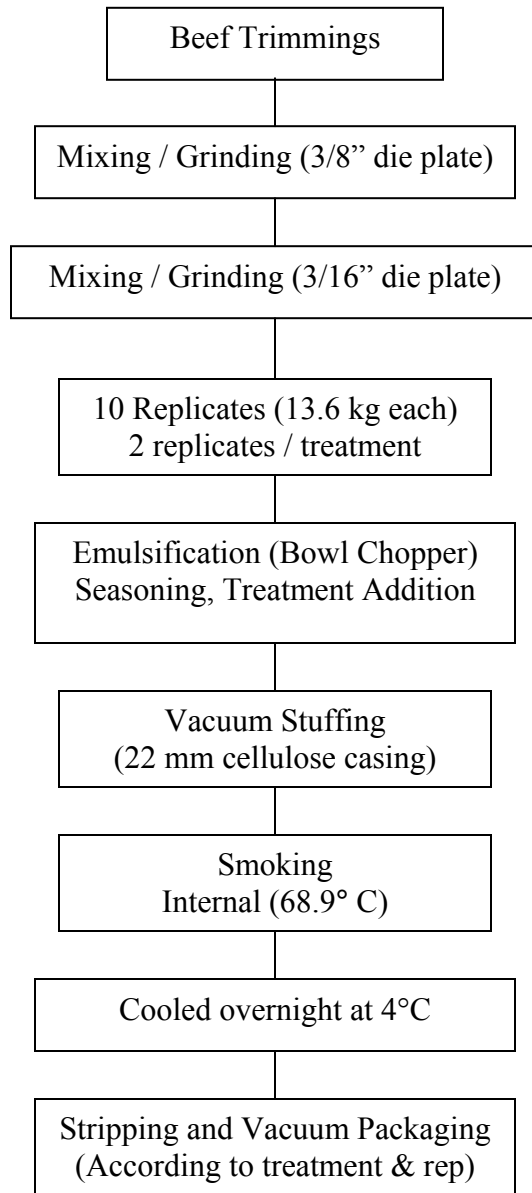
Figure 8. Frankfurter Inoculation Flowchart Diagram

Frankfurters
36 per replicate
72 per treatment
360 total

Inoculation on sterile Al⁻ sheet
10 μL of a 10^9 $\log_{10}$ CFU/mL of LM
Brie 1 (1500 μg/mL streptomycin
sulfate-resistant)

Bacterial attachment period
5 min at 25° C

Aseptic placement into
vacuum pouch

Vacuum packaging

Storage at 4° C
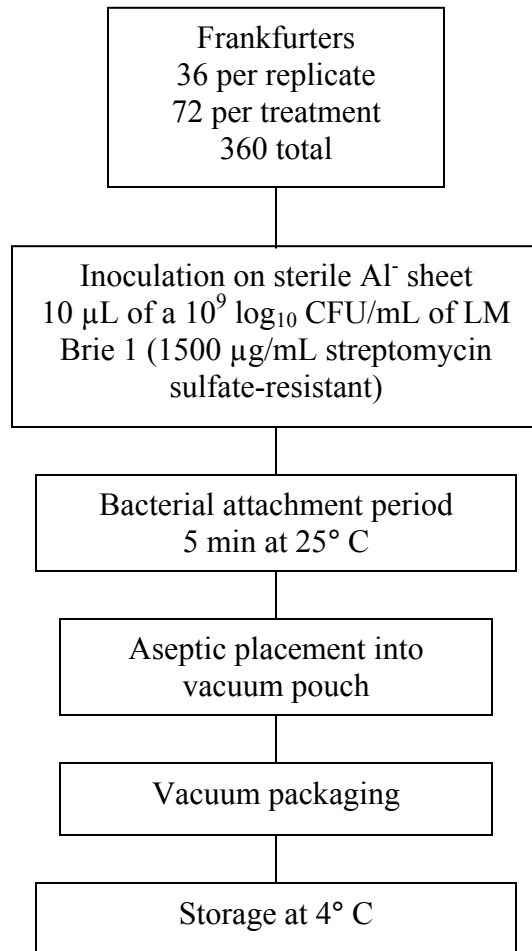
Figure 9. Shelf-life Enumeration Flowchart Diagram

```
                    ┌──────────────────────────┐
                    │    6 Frankfurters per     │
                    │        Treatment          │
                    │     (3 per replicate)     │
                    └──────────────────────────┘
                                 │
        ┌───────────────────────────────────────────────────┐
        │            Each Treatment Replicate                │
        │   (+ 50 mL 1X Phosphate Buffered Saline (PBS))     │
        └───────────────────────────────────────────────────┘
                                 │
                    ┌──────────────────────────┐
                    │        Shaken 1 min       │
                    └──────────────────────────┘
                                 │
                    ┌──────────────────────────┐
                    │       Serial Dilutions    │
                    └──────────────────────────┘
                       /                     \
    ┌────────────────────────────┐   ┌────────────────────────────┐
    │ Aerobic Plate Counts (APC)  │   │    Psychrotrophs (PSY)      │
    │  Spiral plated onto Plate   │   │  Spiral plated onto Plate   │
    │      Count Agar (PCA)       │   │      Count Agar (PCA)       │
    └────────────────────────────┘   └────────────────────────────┘
                  │                                 │
    ┌────────────────────────────┐   ┌────────────────────────────┐
    │  Incubation 24 – 48 h at 37°C │ │  Incubation 10 days at 4°C  │
    └────────────────────────────┘   └────────────────────────────┘
                  │                                 │
    ┌────────────────────────────┐   ┌────────────────────────────┐
    │  Enumerated with QCount®    │   │  Enumerated with QCount®    │
    │     Machine/Software        │   │     Machine/Software        │
    │   (log10 CFU/frank)         │   │   (log10 CFU/frank)         │
    └────────────────────────────┘   └────────────────────────────┘
```

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Figure 10. *Listeria monocytogenes* Enumeration Flowchart Diagram

```
┌─────────────────────────┐
│   6 Frankfurters per     │
│       Treatment          │
│    (3 per replicate)     │
└─────────────────────────┘
             │
┌─────────────────────────────────────┐
│ Aseptic transfer of individual franks│
│      to Whirl-Pak™ filter bags        │
└─────────────────────────────────────┘
             │
┌─────────────────────────┐
│    50 mL 1X Phosphate    │
│ Buffered Saline (PBS) added │
└─────────────────────────┘
             │
┌─────────────────────────┐
│      Shaken 1 min        │
└─────────────────────────┘
             │
┌─────────────────────────┐
│     Serial Dilutions     │
└─────────────────────────┘
             │
┌─────────────────────────┐
│ Spiral plated onto Brain Heart │
│ Infusion Agar (BHI) + 1500 │
│ μg/mL streptomycin sulfate │
└─────────────────────────┘
             │
┌─────────────────────────┐
│ Incubation 24 – 48 h at 35°C │
└─────────────────────────┘
             │
┌─────────────────────────┐
│  Enumerated with QCount® │
│    Machine/Software      │
│   (log₁₀ CFU/frank)      │
└─────────────────────────┘
```

Enumerated with QCount$^{®}$ Machine/Software ($\log_{10}$ CFU/frank)

Table 1. Sensory panelist results for appearance, flavor, texture, juiciness, and overall of beef frankfurters either formulated without acid marinades (Control) or formulated with sodium lactate (SL; 2%), potassium lactate (PL; 2%), sodium citrate (SC; 0.75%), and sodium lactate/sodium diacetate combination (SL/SD; 2%/0.25%) by storage period (week).

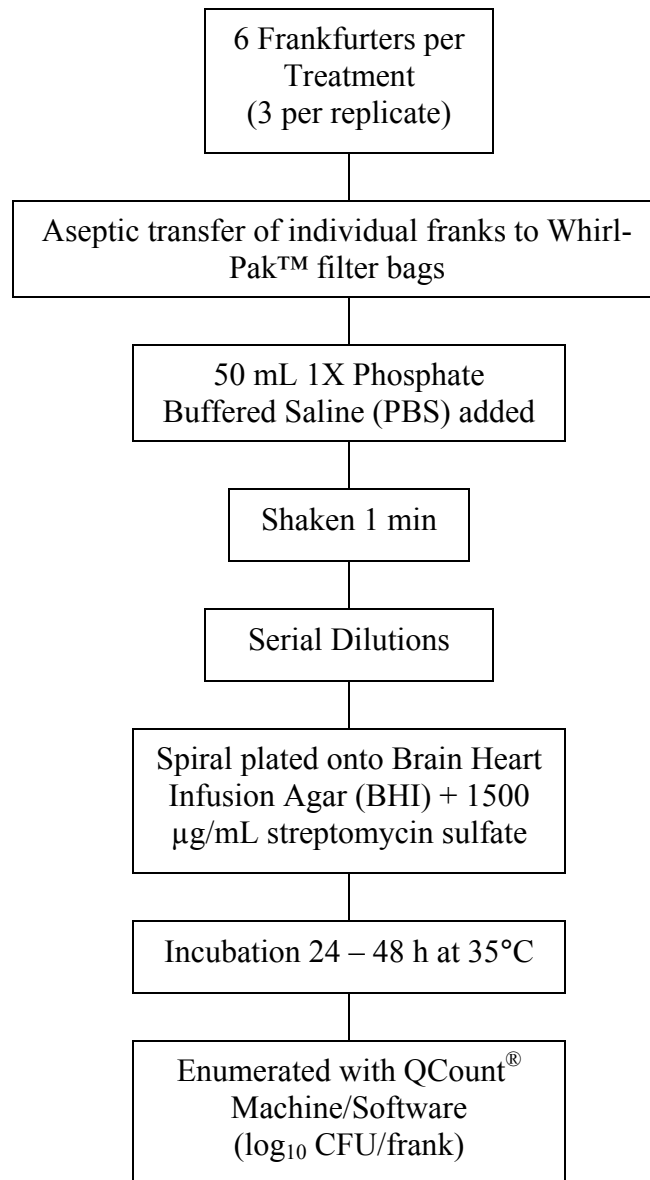| Storage Period	Treatment	Appearance	Flavor	Texture	Juiciness	Overall
	Control	$2.57^b \pm 1.05$	$2.59^{bc} \pm 1.40$	$3.01^b \pm 0.98$	$2.69^b \pm 1.13$	$2.83^b \pm 1.21$
	SL	$2.61^b \pm 1.24$	$2.62^{bc} \pm 1.40$	$2.80^b \pm 1.56$	$2.58^b \pm 1.38$	$2.89^b \pm 1.38$
Week 1	PL	$2.68^b \pm 1.46$	$2.47^c \pm 1.35$	$2.89^b \pm 1.08$	$2.80^b \pm 1.36$	$2.72^b \pm 1.37$
	SC	$2.49^b \pm 1.16$	$2.89^b \pm 1.12$	$2.74^b \pm 1.02$	$2.73^b \pm 1.07$	$2.76^b \pm 1.03$
	SL/SD	$\mathbf{5.54^a \pm 1.65}$	$\mathbf{6.71^a \pm 1.56}$	$\mathbf{6.06^a \pm 1.22}$	$\mathbf{5.27^a \pm 1.77}$	$\mathbf{6.10^a \pm 1.68}$
	Pooled SEM	0.17	0.14	0.18	0.18	0.17
	Control	$2.73^a \pm 1.22$	$2.89^a \pm 1.24$	$2.76^a \pm 1.14$	$2.96^a \pm 1.04$	$2.83^a \pm 1.10$
	SL	$2.82^a \pm 1.24$	$2.75^a \pm 1.41$	$2.90^a \pm 1.32$	$2.49^b \pm 0.92$	$2.89^b \pm 1.37$
Week 2	PL	$2.80^a \pm 1.28$	$2.69^a \pm 1.28$	$2.87^a \pm 1.07$	$2.71^{ab} \pm 0.97$	$2.80^a \pm 1.14$
	SC	$2.93^a \pm 1.28$	$2.70^a \pm 1.21$	$2.84^a \pm 0.94$	$2.71^{ab} \pm 0.88$	$2.69^a \pm 1.00$
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.17	0.15	0.18	0.13	0.16

[a,b,c] Mean values bearing different superscript letters within each storage period and treatment are significantly different ($P \leq 0.05$). Higher values indicate less acceptable panelist results. SL/SD data not collected weeks 2 through 12.

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Table 1. Continued

Storage Period	Treatment	Appearance	Flavor	Texture	Juiciness	Overall
	Control	2.55^b±0.95	2.72^a±1.09	2.98^a±1.10	2.69^{ab}±1.07	2.81^{ab}±1.03
	SL	2.91^{ab}±1.39	2.86^a±1.51	3.19^a±1.25	2.58^{ab}±0.91	3.07^a±1.37
Week 3	PL	2.56^b±1.04	2.49^a±1.13	2.74^a±1.15	2.37^b±0.88	2.58^b±1.15
	SC	3.00^a±1.12	2.74^a±1.46	2.97^a±1.03	2.79^a±1.01	2.95^{ab}±1.17
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.15	0.15	0.18	0.13	0.16
	Control	2.30^b±0.89	2.54^a±1.09	2.71^a±1.04	2.54^b±0.89	2.59^a±0.91
	SL	2.32^b±0.97	2.32^a±1.07	2.59^a±0.87	2.43^b±0.85	2.36^a±0.92
Week 4	PL	2.29^b±0.91	2.43^a±1.12	2.63^a±1.20	**2.11^a±0.78**	2.41^a±0.97
	SC	**2.80^a±1.15**	2.54^a±1.06	2.54^a±0.93	2.52^b±0.87	2.50^a±0.89
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.13	0.14	0.15	0.11	0.12
	Control	2.56^a±0.95	2.61^a±1.43	2.89^a±1.10	2.63^{ab}±0.98	2.75^{ab}±1.25
	SL	2.51^a±0.94	2.48^a±1.17	2.66^a±1.09	2.36^{bc}±0.85	2.58^{ab}±1.09
Week 5	PL	2.54^a±1.12	2.38^a±1.21	2.53^a±1.14	2.15^c±0.96	2.44^b±1.18
	SC	2.73^a±0.91	2.78^a±1.15	2.76^a±1.13	2.86^a±0.96	2.91^a±1.02
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.13	0.15	0.16	0.12	0.15

[a,b,c]Mean values bearing different superscript letters within each storage period and treatment are significantly different ($P \leq 0.05$). Higher values indicate less acceptable panelist results. SL/SD data not collected weeks 2 through 12.

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Table 1. Continued

Storage Period	Treatment	Appearance	Flavor	Texture	Juiciness	Overall
	Control	2.46^a±0.82	2.69^{ab}±1.55	3.13^a±0.99	2.58^a±0.96	2.86^a±1.30
	SL	2.50^a±1.27	2.38^b±1.47	2.83^a±1.12	2.57^a±0.94	2.59^a±1.18
Week 6	PL	2.87^a±1.41	2.90^a±1.44	3.03^a±1.26	2.71^a±1.29	3.01^a±1.35
	SC	2.78^a±1.12	2.61^{ab}±1.39	2.83^a±0.98	2.80^a±1.01	2.82^a±1.23
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.17	0.16	0.21	0.15	0.18
	Control	2.78^{ab}±1.05	2.73^a±1.31	2.81^a±1.31	2.66^{ab}±0.99	2.80^a±1.31
	SL	2.66^b±0.95	2.66^a±1.24	3.02^a±1.02	2.86^a±1.05	2.72^a±0.93
Week 7	PL	3.14^a±1.33	2.80^a±1.35	3.19^a±1.37	2.49^b±0.94	2.91^a±1.37
	SC	2.85^{ab}±1.13	2.57^a±1.23	2.83^a±0.97	2.88^a±1.08	2.78^a±1.16
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.15	0.16	0.17	0.13	0.16
	Control	2.62^a±1.11	2.64^a±1.24	2.82^a±1.21	2.51^a±0.98	2.70^a±1.12
	SL	2.80^a±1.27	2.71^a±1.47	2.99^a±1.26	2.36^a±0.97	2.88^a±1.42
Week 8	PL	2.81^a±1.13	2.85^a±1.33	3.02^a±1.14	2.52^a±0.99	3.07^a±1.36
	SC	2.86^a±1.26	2.82^a±1.65	3.14^a±1.08	2.64^a±1.12	3.09^a±1.43
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.16	0.16	0.19	0.13	0.18

[a,b,c]Mean values bearing different superscript letters within each storage period and treatment are significantly different ($P \leq 0.05$). Higher values indicate less acceptable panelist results. SL/SD data not collected weeks 2 through 12.

Table 1. Continued

Storage Period	Treatment	Appearance	Flavor	Texture	Juiciness	Overall
Week 9	Control	-----	-----	-----	-----	-----
	SL	-----	-----	-----	-----	-----
	PL	-----	-----	-----	-----	-----
	SC	-----	-----	-----	-----	-----
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	-----	-----	-----	-----	-----
Week 10	Control	$2.82^a \pm 1.49$	$2.92^a \pm 1.50$	$3.21^a \pm 1.24$	$2.97^a \pm 1.17$	$3.22^a \pm 1.52$
	SL	$2.77^a \pm 1.32$	$2.57^a \pm 1.45$	$2.82^a \pm 1.28$	$2.29^b \pm 0.98$	$2.73^a \pm 1.24$
	PL	$2.74^a \pm 1.34$	$2.52^a \pm 1.35$	$2.78^a \pm 1.13$	$2.35^b \pm 1.23$	$2.68^a \pm 1.42$
	SC	$2.51^a \pm 1.09$	$2.83^a \pm 1.62$	$3.05^a \pm 1.12$	$2.70^{ab} \pm 0.92$	$2.95^a \pm 1.28$
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.19	0.18	0.22	0.16	0.20
Week 11	Control	$2.90^a \pm 1.09$	$3.12^a \pm 1.33$	$3.33^a \pm 1.40$	$3.17^a \pm 1.39$	$3.38^a \pm 1.47$
	SL	$3.23^a \pm 1.44$	$2.86^a \pm 1.28$	$3.20^a \pm 1.27$	$2.64^b \pm 1.12$	$3.05^a \pm 1.32$
	PL	$3.31^a \pm 1.34$	$3.18^a \pm 1.30$	$3.17^a \pm 1.52$	$2.54^b \pm 1.06$	$3.35^a \pm 1.58$
	SC	$3.09^a \pm 1.26$	$2.96^a \pm 1.25$	$2.93^a \pm 1.26$	$2.91^{ab} \pm 1.08$	$3.11^a \pm 1.33$
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.17	0.18	0.17	0.15	0.19

[a,b,c] Mean values bearing different superscript letters within each storage period and treatment are significantly different ($P \leq 0.05$). Higher values indicate less acceptable panelist results. Data not collected week 9 because of low participant numbers. SL/SD data not collected weeks 2 through 12.

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Table 1. Continued

Storage Period	Treatment	Appearance	Flavor	Texture	Juiciness	Overall
	Control	2.76^a±1.27	3.11^a±1.53	3.36^a±1.31	3.05^b±1.25	3.44^a±1.57
	SL	3.12^a±1.37	2.88^a±1.33	3.05^a±1.21	**2.45^a±0.95**	3.13^a±1.28
Week 12	PL	3.20^a±1.53	3.11^a±1.30	3.10^a±1.50	2.85^b±0.93	3.23^a±1.45
	SC	3.18^a±1.27	2.98^a±1.36	3.13^a±1.27	2.87^b±1.26	3.15^a±1.26
	SL/SD	-----	-----	-----	-----	-----
	Pooled SEM	0.17	0.17	0.18	0.14	0.18

[a,b,c]Mean values bearing different superscript letters within each storage period and treatment are significantly different ($P \leq 0.05$). Higher values indicate less acceptable panelist results. SL/SD data not collected weeks 2 through 12.

APPENDICES

A. Smoke Cycle

B. Sensory Panel Information Sheet

C. Example Sensory Panelist Evaluation Sheet

D. Photograph of "Fat caps" of pre-stripped, fully-cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.

E. Photograph of "Fatted-out", pre-stripped, fully-cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.

F. Photograph of "Fatted-out", post-stripped, fully-cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.

APPENDIX A

1. Smokehouse cycle for beef frankfurters, dry bulb and wet bulb temperatures and time.

#	TYPE	TIME	SMK	INT	DRY	WET	%RH	DPT	D	F
1	COOK	030	OFF	199	140	000	000	000	A	H
2	COOK	020	NAT	199	150	000	000	000	A	H
3	COOK	015	NAT	199	165	130	039	127	A	H
4	COOK	010	NAT	199	175	153	058	151	A	H
5	COOK	999	OFF	165	180	152	050	149	A	H
6	SHWR	999	OFF	095	000	000	100	000	0	H

APPENDIX B

SENSORY PANEL INFORMATION SHEET
For Study Entitled
Evaluation of Beef Frankfurters

You are invited to participate in a research study to determine consumer acceptability of beef frankfurters. This study is being conducted by Dr. Shelly McKee, faculty in the Department of Poultry Science and Jordan Bowers in the Department of Poultry Science at Auburn University. We hope to obtain valuable information regarding consumer preferences and responses to several different meat samples. You were selected as a possible participant because you are an Auburn University employee and/or student and are $\geq$ 19 years of age and represent the general consumer market we hope this product will reach.

The products are beef. If you have any sensitivity to beef meat products you should not participate in this study.

If you decide to participate, we will be asking you to try samples of beef frankfurters and evaluate them based on appearance, flavor, texture, juiciness, and overall acceptability using the scales provided on the form that will rate each attribute on a scale from Greatly Like to Greatly Dislike. All deli loaf and frankfurter samples have been cooked to an internal temperature of 72 °C. Each sensory panel that you participate in during this study will take approximately 5-10 minutes to complete.

Any information obtained during this study will remain anonymous. Information collected through your participation may be (i.e., used to fulfill an educational requirement (dissertation), published in a professional journal, and/or presented at a professional meeting; will aid in the further development of this product in order to get it onto the market, etc.). You may withdraw from participation at any time, without penalty, however, after you have provided anonymous information you will be unable to withdraw your data, since there will be no way to identify individual information.

Your decision whether or not to participate will not jeopardize your future relations with Auburn University or Jordan Bowers of the Department of Poultry Science.

I you have any questions we invite you to ask them now. If you have questions later, please feel free to contact Jordan Bowers at (334) 844-8479 or bowerjw@auburn.edu or Dr. Shelly McKee at (334) 844-2765 or mckeesr@auburn.edu or IRBChair@auburn.edu.

HAVING READ THE INFORMATION PROVIDED, YOU MUST DECIDE WHETHER TO PARTICIPATE IN THIS RESEARCH PROJECT. IF YOU DECIDE TO PARTICIPATE, THE DATA THAT YOU PROVIDE WILL SERVE AS YOUR AGREEMENT TO DO SO.

_____   _____
Investigator's Signature   Date   Co-investigator's Signature   Date

APPENDIX C

Sensory Evaluation of Beef Frankfurters

Sample #: _____   Date: _____

You will be given a sample of each frankfurter. Please evaluate the sample for the characteristics listed below. Indicate your degree of liking according to the scales below. In addition, please include any comments you have about this product.

Appearance
_____ Like Extremely
_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much
_____ Dislike Extremely

Flavor
_____ Like Extremely
_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much
_____ Dislike Extremely

Texture

_____ Like Extremely
_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Dislike Slightly
_____ Dislike Moderately
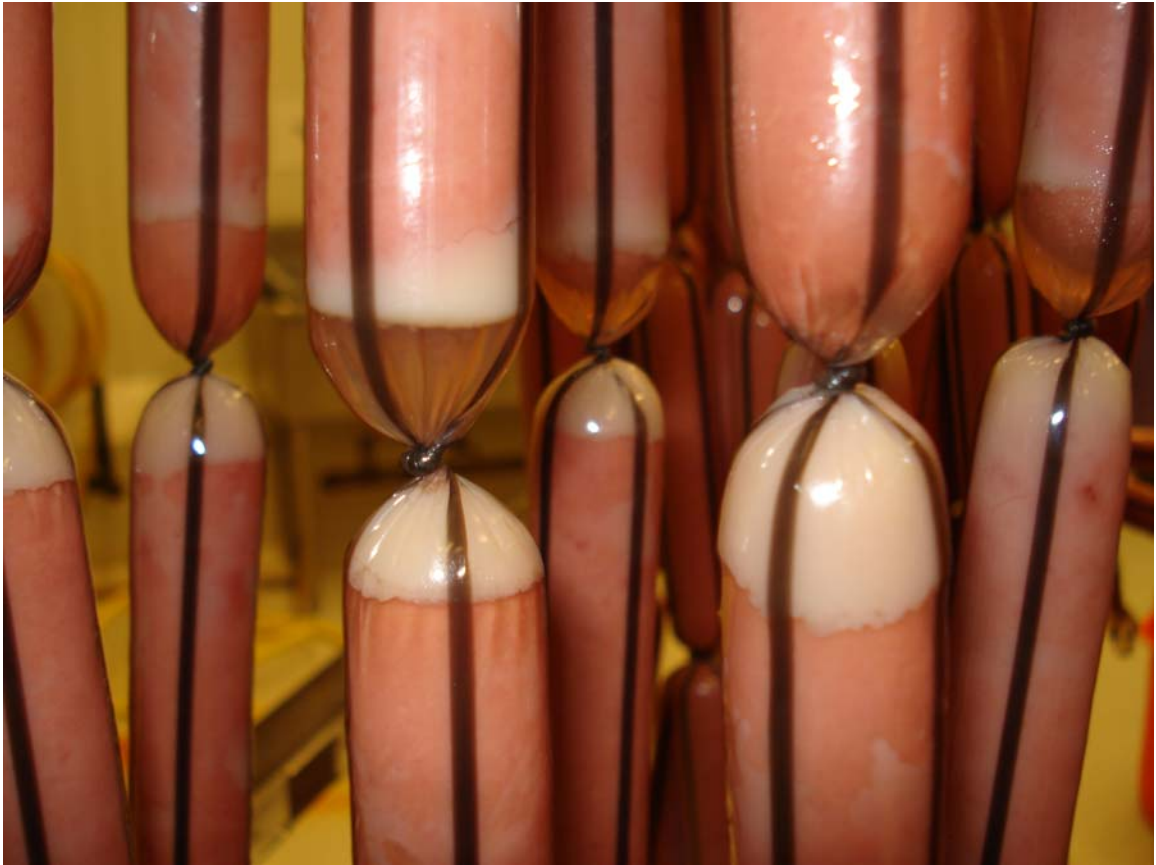_____ Dislike Very Much
_____ Dislike Extremely

Juiciness
_____ Extremely Moist
_____ Very Moist
_____ Moderately Moist
_____ Slightly Moist
_____ Slightly Dry
_____ Moderately Dry
_____ Very Dry
_____ Extremely Dry

Overall Acceptability
_____ Like Extremely
_____ Like Very Much
_____ Like Moderately
_____ Like Slightly
_____ Dislike Slightly
_____ Dislike Moderately
_____ Dislike Very Much
_____ Dislike Extremely

Comments:

Photograph of "Fat caps" of pre-stripped, fully-cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.

Photograph of "Fatted-out", pre-stripped, fully-cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.

Photograph of "Fatted-out", post-stripped, fully cooked beef frankfurters treated with 2% sodium lactate (SL) and 0.25% sodium diacetate (SD) combination.