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Organic Acid Formulation and Dip to Control Listeria monocytogenes in Hot Dogs

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Abstract: Commercially, hotdogs are handled after the cooking potentially causing contamination. Since consumers may eat hotdogs without reheating, the presence of *Listeria Monocytogenes* (LM) is a concern. In this study, treatments with organic acids in the raw product and as a post-cook dip were evaluated for their ability to suppress the growth of LM. Beef hotdogs were formulated with organic acids, cooked, cooled, inoculated with LM and then dipped in organic acid treatments. Treatments included: 1) Potassium Lactate (PL) in the formulation and Sodium Lactate (SL) in the dip, 2) PL in the formulation and SL with Sodium Diacetate (SD) in the dip, 3) SL and SD in the formulation and SL in the dip and 4) SL and SD in the formulation and SL and SD in the dip. Positive (inoculated) and negative (non-inoculated) controls were formulated with no organic acids and dipped in distilled water. All hotdogs were stored at 4°C and the number of LM was evaluated on day 0, 7, 14, 21, 28, 42 and 56. When compared to the positive control, all treatments resulted in significantly decreased LM numbers over time. The SL/SD with SL dip and SL/SD with SL/SD dip were the most effective treatments.

Key words: Listeria monocytogenes, organic acids, hot dogs, lactate, sodium diacetate

INTRODUCTION

Listeria Monocytogenes (LM) is a foodborne bacterium that causes listeriosis, a serious infection that can result from eating food contaminated with the bacteria. There has been an estimated 2,500 illnesses each year, 500 of these resulting in death (CDC, 2005).

Listeria monocytogenes is able to grow and survive under a wide range of temperatures (-1.5 to 45°C) and pH (4.3 to 9.4) (Marc et al., 2002). Because Ready-to-Eat (RTE) products are refrigerated and not usually reheated before consuming, the organism is a potential food safety concern (Johnson et al., 1990). Of the products contaminated with LM, it is assumed that the presence of the pathogen is due to recontamination after cooking. possibly as a result of environmental contamination (Johnson et al., 1990). The contamination of fully cooked products may occur during handling (slicing or packaging) or possibly from cross-contamination (Johnson et al., 1990). Efforts to decontaminate RTE products post-cooking include in-package thermal pasteurization and irradiation and antimicrobial additives (Zhu et al., 2005). By exposing LM to a combination of several additives as a multi hurdle approach, LM can be inhibited in products without adversely affecting sensory properties (Juncher et al., 2000; Marc et al., 2002).

Sodium and potassium lactate, which are derived from lactic acid that is naturally present in animal tissue, prevent bacteria from reproducing by increasing the lag phase or dormant phase of microorganisms (De Vegt,

1999). Sodium diacetate is also used in the food industry as a flavor and antimicrobial agent (Vogel *et al.*, 2006).

Sodium and potassium lactate and sodium diacetate can be incorporated into meat and poultry products to lessen the growth of some pathogenic bacteria including: Listeria, Salmonella, Staphylococcus aureus and Clostridium (De Vegt, 1999). These ingredients work by lowering the pH and water activity of the product, providing an environment that has an adverse effect on the growth of bacteria (Doyle, 1999; De Vegt, 1999). In this study, a multi-hurdle approach, combining Generally Recognized As Safe (GRAS) ingredients in the raw product and as a post-cook dip, was evaluated for ability to suppress the growth of LM in hot dogs.

MATERIALS AND METHODS

Organic acids were combined in the raw product and as a post-cook dip for a multi-hurdle approach to determine the effect they have on the growth of LM on beef hot dogs. The treatments are shown in Table 1. All treatments included a standard industry antimicrobial cocktail of sodium chloride (1.5%), sodium tripolyphosphate (0.45%), sodium nitrite (156 ppm) and hot dog seasoning (6.25% per 11.34 kg).

Inoculum preparation: A streptomycin-resistant *Listeria monocytogenes* strain stored at-80°C in 10% glycerol was used for all experiments. Inocula were prepared by

Table 1: Formulation for the marinade ingredients and post-cook dip for beef hot dogs

Treatments	Antimicrobial in formulation	Post-cook dip
1	PL ^b (2%)	SL (3.6%)
2	PL (2%)	SL (3.6%), SD (0.25%)
3	SL ^c (2%), SD ^d (0.25%)	SL (3.6%)
4	SL (2%), SD (0.25%)	SL (3.6%), SD (0.25%)
Control ^a	Salte (1.5%), STPP (0.45%), Na nitrite (156 ppm),	Distilled water
	Seasoning ^h (6.25%/11.34 kg)	

Salt (1.5%), STPP (0.45%), sodium (Na) nitrite (156 ppm) and hot dog seasoning (6.25% per 11.34 kg) were added in all formulations. Positive (inoculated) and negative (non-inoculated) control.

reviving from frozen storage and growing in ml tubes of BHI broth (Oxoid Ltd., Hampshire, UK), which were incubated at 37°C overnight. Three replicate aliquots of 1.0 ml were taken from the culture tube and placed into three separate 9 ml tubes of fresh BHI broth, which were also incubated overnight at 37°C. Cell numbers in suspension were enumerated by spread plating onto duplicate BHI agar plates (Difco, Fisher Scientific Co. LLC, Pittsburgh, PA) with the addition of 1500 mg/ml Streptomycin Sulfate Salt (Sigma-Aldrich Inc., St. Louis, MO) and were incubated overnight at 37°C. The cell suspension was determined to be 1.47x10° CFU/ml and 2.05x10° CFU/ml for trials 1 and 2, respectively.

Processing hot dogs: All processing was conducted using sterile trays, racks and pans. A total of 27.22 kg of ground beef and 27.22 kg of trimmings were purchased from Excel (beef trimmings 50/50 and 93/7 Coarse Grind Ground Beef, Cargill Meat Solutions Corporations, Wichita, Kansas). Ingredients for the two control emulsions and the four treated emulsions were based on 6.80 kg (50% lean and 50% trim). Final concentrations of the antimicrobial ingredients were as follows:

- Sodium chloride (1.5%) (Morton Salt, Morton International Inc., Morton Salt, Chicago, IL)
- Sodium Tripolyphosphate (0.45%) (Innophos, Cranbury, NJ)
- Sodium Nitrite (156 ppm) (A.C. Legg, Inc. Birmingham, AL)
- Hot dog seasoning (6.25% per 11.34 kg) (A.C. Legg, Inc., Birmingham, AL)
- Potassium Lactate (2%) (60% w/w, City Chemical LLC., West Haven, CT)
- Sodium Lactate (2%) (60% w/w, Fisher Scientific Co. LLC., Pittsburgh, PA)
- Sodium Diacetate (0.25%) (Spectrum Chemical MFG. Corp. Gardena, CA)

The pH (Accument AB15 Plus pH Meter, Fisher Scientific Inc., Rockford, IL) was measured for the antimicrobial ingredients and pre-treated raw product. For each formulation, 3.40 kg of trimmings were cubed and chopped (64108 Koch Bowl Chopper, Kansas City, Missouri) for one to three min. After chopping, trimmings were removed, 3.40 kg of ground beef was added to the bowl and allowed to chop for one to two min. Of the 20% marinade, 50% was ice. Half of the ice was added along with ingredients, separately by treatment, for one to two min. Finally, trimmings were added back in with the remaining ice and allowed to chop for four to eight min. The pH of the post-marinated (antimicrobial ingredients) raw product was then determined. Once an emulsion was formed, beef hot dogs were vacuum stuffed (68400 Handtmann VF 50, Albert Handtmann Maschinenfabrik, Biberach/Riss, Germany) by treatment into fibrous casings (EZ Peel Fibrous Casing, Viskase Companies, Inc., Willowbrook, IL) and cooked in a smokehouse (Alkar Smokehouse, DEC Internation, Lodi, WI) to an internal temperature of 74°C measured by Multitrip (Multi use temperature recorder, Temprecord, Modesto, CA). Following cooking, hot dogs were then cooled to an internal temperature of 4°C, casings were removed and hot dogs were cut into 15.24 cm links.

Microbiological analysis: Each link was inoculated by dripping streptomycin-resistant LM (10⁷ cells LM per link resulting in 10⁵ inoculum on product) onto the surface and spreading with a sterile plastic inoculating loop. Links were allowed to air dry (5 min) on autoclaved foil before dipping them into the post-cook marinade dip. Eight beakers, each with 500 ml, were prepared for each post-cook treatment dip. The pH and water activity (TH-500 A_w Sprint, Novasina, Pfäffikon, Switzerland) was noted for each treatment. After immersion for 60 sec, 5 links were left on sterile foil and allowed to air dry at room temperature for approximately 15 to 20 min. Three links for every treatment were placed together in a whirl-

^bPotassium Lactate (PL), 60% w/w, City Chemical LLC., West Ha∨en, CT.

[°]Sodium Lactate (SL), 60% w/w, Fisher Scientific Co. LLC., Pittsburgh, PA.

dSodium diacetate, Spectrum Chemical MFG. Corp. Gardena, CA.

eSalt, Morton Salt, Morton International Inc., Morton Salt, Chicago, IL.

¹Sodium tripolyphosphate, Innophos, Cranbury, NJ.

⁹Sodium nitrite, A.C. Legg, Inc., Birmingham, AL.

^hHot dog seasoning, A.C. Legg, Inc., Birmingham, AL

pak bag (21 links per treatment, 7 bags per treatment, totaling 126 links and 42 bags) (Whirl-pak Bags, Nasco, Fort Atkinson, WI) and placed at 4°C for 0, 7, 14, 21, 28, 42, or 56 d prior to analysis.

Non-inoculated negative control links were dipped in sterile water for 60 sec, allowed to dry 15 to 20 min, packaged in whirl-pak bags and immediately placed at 4°C. Positive control links were inoculated with LM, allowed to dry 5 min, dipped in sterile water for 60 sec, allowed to dry for approximately 15 to 20 min, packaged and stored at 4°C. The treated links and positive and negative control links were tested for LM on day 0 time point to ensure proper inoculation was attained.

For each testing time point, one link was removed from each bag, placed in a filter stomacher bag (Filtered Homogenizer Bags, 3M, St. Paul, MN) with 50 ml of sterile Phosphate Buffered Saline (PBS) (Pierce, Thermo Fisher Scientific Inc., Rockford, IL) and homogenized (400 Circulator Stomacher, Seward, England) for 2 min at 230 RPMs. This process was completed for all three samples. The samples were then further diluted, 1 ml into 9 ml of sterile PBS. Because the samples contained fat and meat particles, they were filtered through a syringe (10 ml syringe, VWR International, Inc., Bristol, CT with a 1.2 µm filter, Minisart single use syringe filter, sterile-EO, non-pyrogenic, Sartorius, Edgeqwood, NY) and dispensed into a sample cup before spiral plating. Dilutions 1 and 4 were plated onto BHI agar with 1500 mg/ml of Streptomycin using a spiral plater (AP 4000 Spiral Biotech, Automated Spiral Plater, Advanced Instrument, Inc., Norwood, MA) with a vacuum (VS2 Spiral Biotech, Vacuum Source, Advanced Instrument, Inc., Norwood, MA) according to manufactures' instructions. Plates were incubated at 35°C for 48 h. Resulting colonies were counted using an automated colony counter (530 Spiral Biotech Automatic Plate Counter, Advanced Instruments, Inc., Norwood MA).

Statistical analysis: The experiment was repeated twice (2 trials) with 3 replications within each trial. No replication x treatment or trial x treatment interactions were noted; therefore, the data was pooled by treatment and day. Data was analyzed by day and over time using Proc GLM procedure of SAS (SAS Institute, 1993). Means were separated by Duncan's Multiple Range test. Significant differences among treatment groups was determined at a level of p<0.05.

RESULTS AND DISCUSSION

Earlier reports show the growth of LM can be inhibited by lowering the pH and water activity, creating an adverse environment (De Vegt, 1999). The pH and water activity data for the antimicrobial formulations and post-cook dips are shown in Table 2. Acids were more effective when used in combination compared to alone in the formulation and in the post-cook dip, the pH was lower. For treatments containing Sodium Lactate (SL), Sodium Diacetate (SD) combination and Potassium Lactate (PL) individually in formulation the pH was 4.97 and 6.95, respectively. The pH was lower for the SL/SD combination (5.32) than for SL alone (6.25) for the postcook dip. Water activity for the dips containing organic acids (SL and SL/SD) was lower than the control, which was sterile distilled water (0.86, 0.85, 1.00, respectively). The number of LM detected on links subjected to the various treatment combinations is shown in Table 3. On day 7, there were no significant differences between the treated links and the positive control. However, the numbers of LM significantly decreased by day 14 in hot dogs containing SL and SD with SL as a dip (SL/SD-SL) and SL and SD both in the marinade and as the dip (SL/SD- SL/SD) when compared to the PL with SL dip (PL-SL) and PL with SL and SD dip (PL-SL/SD) treatments and the positive control. There was a similar trend on day 21 as day 14 where the colony counts for the SL/SD-SL and SL/SD-SL/SD treatments were significantly lower than the other treatments. These

Table 2: Antimicrobial pH, pre-and post-marination pHs of the raw beef hot dogs and dip solution pH and A_w for the control and

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Treatments	Antimicrobial pH	Pre-marination pH	Post-marination pH	Antimicrobial A _w
Ingredients				
Pla	6.95	5.61	5.80	
SL/SD ^b	4.97	6.08	6.06	
Control [€]	7.00	5.68	5.62	
Dips				
SLd	6.25			0.86
SL/SD ^e	5.32			0.85
Control ^f	7.00			1.00

Potassium lactate (2%), salt (1.5%), STPP (0.45%), sodium nitrite (156 ppm), hot dog seasoning (6.25% per 25 lb) and water.

^bSodium lactate (2%), sodium diacetate (0.25%), salt (1.5%), STPP (0.45%), sodium nitrite (156 ppm), hot dog seasoning (6.25% per 25 lb) and water.

[°]Salt (1.5%), STPP (0.45%), sodium nitrite (156 ppm), hot dog seasoning (6.25% per 25 lb) and water.

^dSodium lactate (3.6%) and water.

[°]Sodium lactate (3.6%), sodium diacetate (0.25%) and water.

^fDistilled water

Table 3: Streptomycin-resistant Listeria monocytogenes (CFU/ml) and standard error of the mean within a day sampled on day 0 to 56 on beef hot dogs (n = 210) stored at 4°C when organic acids were applied in the raw product combined with a post-cook dip

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Treatments	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56
1 ^d	4.44°±0.13	4.54°±0.19	4.15°±0.06	4.16°±0.15	3.93 ^b ±0.19	3.80 ^b ±0.15	3.11b±0.29
2 ^e	4.37 ^{ab} ±0.12	4.44°±0.09	4.42ab±0.11	4.02°±0.15	4.07b±0.13	3.53b±0.10	1.90°±0.03
3^f	4.26ab±0.11	4.27°±0.16	3.67°±0.12	1.40b±0.11	<1.00°	<1.00°	<1.00°
4 ^g	4.22b±0.12	4.22°±0.15	3.71°±0.13	1.70°±0.10	<1.00°	<1.00°	<1.00°
Control +h	4.31ab±0.10	4.51°±0.17	4.63°±0.17	4.57°±0.12	4.56°±0.10	4.64°±0.06	5.70°±0.12

Salt (1.5%), STPP (0.45%), sodium (Na) nitrite (156 ppm) and hot dog seasoning (6.25% per 11.34 kg) were added in all formulations.

Table 4: Streptomycin-resistant *Listeria monocytogenes* (CFU/ml) and standard error of the mean over time sampled on day 0 to 56 on beef hot dogs (n = 210) stored at 4°C when organic acids were applied in the raw product combined with a post-cook dip

Treatment	Day 0	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56
1 ^d	4.44°±0.13	4.54°±0.19	4.15°±0.06	4.16°±0.15	3.93ab±0.19	3.80ab±0.15	3.11b±0.29
2e	4.37°±0.12	4.44°±0.09	4.42°±0.11	4.02°±0.15	4.07°±0.13	3.53°±0.10	1.90 ^b ±0.03
3 ^f	4.26°±0.11	4.27°±0.16	3.67°±0.12	1.40b±0.11	<1.00°	< 1.00°	<1.00°
4 ^g	4.22°±0.12	4.22°±0.15	3.71°±0.13	1.70b±0.10	<1.00°	<1.00°	<1.00°
Control+h	4.31b±0.10	4.51b±0.17	4.63b±0.17	4.57b±0.12	4.56b±0.10	4.64b± 0.06	5.70°±0.12

Salt (1.5%), STPP (0.45%), sodium (Na) nitrite (156 ppm) and hot dog seasoning (6.25% per 11.34 kg) were added in all formulations.

results are similar to that of Blom *et al.* (1997) whom found that when potassium lactate was utilized individually, the inhibition was not as large as the combined treatments (sodium lactate and sodium acetate)

On day 28, 42 and 56, hot dogs with SL/SD-SL and SL/SD-SL/SD treatments had no detectable LM. Also at these time points, the numbers of LM on links treated with PL-SL and PL-SL/SD was significantly lower than the positive control. After day 28, the number of LM on positive control hot dogs was higher than all other treatments. Therefore, adding organic acids was better than the standard industry control formulation at controlling LM. The SL/SD-SL and SL/SD-SL/SD treatments were best at controlling LM after 14 days. As stated in Stekelenburg (2003), some studies conclude that when organic acids are individually added to hot dog formulation, their ability to inhibit LM is not as significant as that of organic acid mixtures.

Stekelenburg (2003) reported similar results in the growth of LM in hot dog sausage. In a solution containing 2% to 3% lactae/diacetate mixture, almost full inhibition of LM was reached during 29 days at 4°C; whereas, some growth was observed in products that only contained potassium lactate (Stekelenburg, 2003).

When each treatment was analyzed through storage time, the data shows interesting trends and effects (Table 4). The number of LM detected on hot dogs with PL-SL and PL-SL/SD treatments were not different from day 0 to day 42; however by day 56 the numbers had significantly decreased to 3.11 CFU/ml and 1.90 CFU/ml, respectively. Treatments containing SL/SD-SL and SL/SD-SL/SD were also not different from day 0 to day 14. Listeria monocytogenes growth started to decrease by day 21 and by day 28 it significantly decreased resulting in <1.00 CFU/ml. Samelis et al. (2002) showed similar results when sodium lactate was added to the formulation for hot dogs. When 1.8% sodium lactate was utilized alone, it permitted LM growth at 35 to 50 days; however, like the current data, lactate combined with sodium acetate and diacetate completely inhibited growth for 120 days of storage at 4°C (Samelis et al., 2002). Those researchers (Samelis et al., 2002). concluded that combinations of GRAS chemical antimicrobials at lower concentrations than allowed for processed meat product, provide better inhibition against LM than the higher concentration of the same antimicrobials used singly.

The pKa values (acid dissociation constant) of sodium lactate, potassium lactate and sodium diacetate are 3.86, 3.86 and 3.58, respectively (Benjamin, 1998; Berg et al., 2002; Vasseur et al., 1999). Phan-Thanh et al. (2000) state that weak organic acids are more deleterious to LM than strong inorganic acids such as hydrochloric acid.

Even though lactic acid does not penetrate the cell membrane efficiently, sodium and potassium lactate is

a,b,cLeast square means within a column with different superscripts differ (p<0.05)

^dPotassium lactate (2%)-sodium lactate (3.6%).

ePotassium lactate (2%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

Sodium lactate (2%) and sodium diacetate (0.25%)-sodium lactate (3.6%).

⁹Sodium lactate (2%) and sodium diacetate (0.25%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

^hControl + - inoculated control dipped in distilled water

^{a,b,c}Least square means within a row with different superscripts differ (p<0.05)

^dPotassium lactate (2%)-sodium lactate (3.6%).

Potassium lactate (2%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

Sodium lactate (2%) and sodium diacetate (0.25%)-sodium lactate (3.6%).

⁹Sodium lactate (2%) and sodium diacetate (0.25%)-sodium lactate (3.6%) and sodium diacetate (0.25%).

hControl + - inoculated control dipped in distilled water

still diffused in the cell affecting the growth of the bacteria (Vasseur *et al.*, 1999). This phenomenon may explain the current data where, the lactic acid that was added in formulation and as a dip to beef hot dogs inhibited LM growth throughout the 56 days.

The numbers of LM did not change on positive control hot dogs from day 0 to day 42 of storage; however, by day 56 LM numbers had significantly increased by 1 log. This could be due to the amount of sodium nitrite and sodium chloride present. Shahamat et al. (1980) conducted a study to determine the efficiency of sodium nitrite for controlling LM. It was concluded that sodium nitrite activity was dependent on interactions with temperature, pH and sodium chloride content (Shahamat et al., 1980). At levels permitted in meat products (156 ppm), sodium nitrite had a significant inhibitory activity only in refrigerated products with at least 3% sodium chloride and a pH of 5.5 or less (Shahamat et al., 1980). Sodium nitrite (156 ppm) and sodium chloride (1.5%) (122.47 g and 17.01 g, respectively) were added in beef hot dog formulation. Exposure of LM to 100 to 200 ppm nitrate resulted in nearly 100% cellular injury (Ngutter and Donnelly, 2003). Ngutter and Donnelly (2003) concluded that increased exposure and concentration of sodium nitrite resulted in an increase in level of injury and repair time.

The SL/SD-SL and SL/SD-SL/SD treatments were most effective in inhibiting LM numbers. Sodium nitrite may have suppressed growth of LM in the control. Utilizing SL and SD in combination in the raw product and as a post-cook dip in the meat and poultry industry, the growth of LM in hot dogs can be suppressed over the extended shelf-life of the product. Further studies should be completed comparing the inhibitory effect of a SL, SD treatment, SL, SA treatment and SL, SD, SA treatment.

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