Effect of Potassium Lactate and Sodium Diacetate Combination to Inhibit *Listeria Monocytogenes* In Low and High Fat Chicken and Turkey Hotdog Model Systems

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Abstract: Effect of potassium lactate (PL) and sodium diacetate (SD) combinations at varying levels were evaluated in low (5%) and high (20%) fat chicken and turkey hotdog model systems. All the samples were surface inoculated with *Listeria monocytogenes* (approximately 4.6 log cfu/g), vacuum packed and stored at 4 °C for 28 days to determine the effective combination of PL and SD and the effect of fat content on the growth inhibition of *L. monocytogenes*. In chicken hotdog samples, maximum growth inhibitions (3.4 log cfu/g) were observed in low fat samples formulated with 3.0% PL and 0.15% SD. In turkey hotdog samples, maximum growth inhibitions (3.3 log cfu/g) were observed in low fat samples formulated with 3.0% PL and 0.2% SD. Effective combination levels determined in low and high fat chicken were 3.0% PL and 0.15% SD, whereas in low and high fat turkey, the effective levels were 3.0% PL and 0.20% SD. Overall, fat content had significant effect (P < 0.05) on growth inhibition as indicated by higher inhibitions in low fat chicken and turkey hotdogs than high fat samples. These results demonstrate that commercial usage levels of PL (2.0%) and SD (0.15%) alone are not sufficient to control *L. monocytogenes* in case of pathogen contamination.

Keywords: Fat content, Hotdogs, Listeria monocytogenes, Potassium Lactate, Sodium Diacetate.

INTRODUCTION

Listeria monocytogenes (L. monocytogenes) is the most common post-processing bacterial contaminant in ready-toeat (RTE) meat and poultry products and has elicited intense concerns to consumers and processors due to recurrent outbreaks resulting in associated product recalls [1]. Among the RTE foods contaminated by L. monocytogenes, deli meats and non-reheated hotdogs poses "very high risk" per serving risk of illness/death [2]. As a result, the U.S. Dept. of Agriculture (USDA) and Food and Drug administration (FDA) maintains a "zero tolerance" (no detectable levels permitted) policy for L. monocytogenes in RTE products [3].

Based on the public health significance and treatment costs, there is a need to develop effective intervention strategies to inhibit or kill the pathogens that would improve the microbiological quality of the meat [4]. Control measures for safety of RTE poultry products should include reducing the risk of contamination as well as inhibiting the growth of pathogens during handling and storage. Conventional chemical antimicrobials such as lactates and diacetates act as bacteriostatic agents against foodborne pathogens including *L. monocytogenes* in meat and poultry products.

Potassium lactate (PL) is a clear syrupy liquid derived from lactic acid and acts as bacteriostatic agent by extending the lag phase or dormant phase of pathogens and thereby prolonging the shelf life of the food products [5]. The specific mechanisms of actions of lactates are: (1) reducing the water activity (a_w) of the product [6] and (2) intracellular acidification [7]. Sodium diacetate (SD) is bactericidal in action against L. monocytogenes by lowering the intracellular pH and thereby significantly inhibiting the growth of the initial bacterial load [8]. Both PL and SD are FDA approved and classified as GRAS (Generally Recognized as Safe) ingredients in RTE meat and poultry products [9]. Maximum permissible levels of lactates (60% lactate solution) and diacetates used in the meat formulations are 4.8% and 0.25% respectively, based on the batch weight of total formulation [10]. However, higher concentration levels of lactates (> 3 %) affect sensory properties [11]. The maximum level for sodium diacetate from a flavor perspective is 0.1 to 0.15%, whereas its inhibitory effects become apparent from 0.125% [12]. At 0.20% concentration, it has a negative effect on odor and taste of the product [5]. Combination of these two antimicrobials have demonstrated enhanced inhibition of the growth of L. monocytogenes on RTE meats during long-term refrigerated storage than when used alone [13].

Antimicrobial activity of any preservative depends on their hydrophilic and hydrophobic properties i.e. solubility in water and fat, distribution in the model system, fat content,

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pH and temperature [14]. Effective combinations of lactates and diacetates varies from one product to another as influenced by difference in meat matrices, formulations (types of meat, moisture and fat content, water activity, pH, salt and nitrite levels), storage temperature and packaging conditions [15, 16]. In addition, in biphasic foods such as hotdogs (oilin-water emulsion), food structure and lipid component may have a controlling influence on growth of the pathogen by its tendency to redistribute chemical components between phases of foods and controlling the concentration of undissociated antimicrobial compounds in the aqueous phase [17]. As the undissociated form of organic acid is lipophilic, less of the undissociated acid or antimicrobial may localize in the aqueous phase and hence can affect their efficacies [18]. Furthermore, between the chicken and turkey meat, variation in protein and fatty acid profiles exist [19].

To our knowledge, there is no published literature on the influence of fat content on the growth of *L. monocytogenes* in presence of lactate and diacetate combinations in chicken and turkey hotdog formulations. Therefore, the main purpose of this study was to determine the effective combination of PL and SD to inhibit the growth of *L. monocytogenes* in surface inoculated low and high fat chicken and turkey hotdog model systems.

MATERIALS AND METHODS

Materials

Mechanically separated chicken and fresh, boneless, skinless chicken breast (Tyson Foods Inc, Springdale, AR, USA) and mechanically deboned turkey (Cargill Meat Solutions, Springdale, AR, USA) were used to prepare low and high fat chicken and turkey hotdogs. Non-meat ingredients for hotdog preparation included salt, sodium tripolyphosphate, dextrose, monosodium glutamate, (Heartland Supp. Co, AR, USA), red pepper, black pepper (Eatem Foods Company, NJ, USA), sodium nitrite (Southern Indiana Butcher Supply, IN), potassium lactate (PURASAL® 60% HiPure P, Purac America, Lincolnshire, IL) and sodium diacetate (Jarchem, NJ, USA). Non-edible casings were used to stuff the emulsified meat (Casings: 30 mm diameter fibrous cellulose casings; E-Z Peel4 Nojax, 30-84 4STR clear, Viskase Corp., Willowbrook, IL, USA). Oxford Listeria selective agar was used to isolate Listeria monocytogenes (EMD Chemicals Inc., Gibbstown, NJ, USA).

Methods

Hotdog Preparation

High fat (20% fat in final product) hotdogs were formulated using mechanically separated chicken or turkey meat, whereas the low fat (5% fat in final product) hotdogs were formulated using ground boneless, skinless chicken breast meat or mechanically separated turkey meat. Non-meat ingredients used in preparation of low and high fat chicken and turkey hotdogs included ice, salt, sodium tripolyphosphate, dextrose, sodium nitrite, dextrose, red and black pepper, and monosodium glutamate. Ground meat was mixed with nonmeat ingredients and varying levels of PL and SD combinations (Fig. 1) to form a homogenous emulsion batter in a bowl chopper (Type K64V-VA, Seydelman, Germany). The meat emulsion was transferred to a sausage stuffer (Friedrich Dick hand stuffer, 15LTR, Germany) with inedible cellulose casings (30 mm diameter) and slid along the horn of the stuffer. This emulsion was stuffed, extruded, pinched and twisted into 6-inch hotdogs links. Hotdogs were placed on cooking sticks in an oven (ALKAR-RapidPak, Inc, Model-1000, Wisconsin, USA) at 82.2 °C until the internal temperature reached 73.8 °C to kill/inactivate all the foodborne pathogens. After cooking, hotdogs were showered with water at 25.5 °C and stored at 4 °C for surface inoculation and storage studies.

Proximate Analysis

Determination of percent moisture (AOAC 2000, method 985.14), protein (AOAC 2000, method 992.15), and fat (AOAC 2000, method 985.15) were conducted in the hotdogs at the start of the experiment. Eight hotdogs (noninoculated) per meat and fat type were homogenized in a food blender (Oster[®] 16-speed blender; Model-6687, Sunbeam Products, Inc., FL, USA) and sampled for moisture, protein and fat analyses in triplicates.

Water Activity (a_w)

Homogenized hotdog samples were spread evenly up to half of the sample cup and positioned inside the vapour chamber of AquaLabTM analyser (Model 3 series, Decagon Devices Inc., Washington DC, USA) at 20 °C to determine the a_w in triplicates.

Residual Nitrite and pH

Residual nitrite (ppm) in hotdogs (meat and fat type) was determined in triplicate samples by colorimetric method (AOAC 2000, method 973.31). For pH determination, frankfurter samples were first stomached for 120 s at 8.0 strokes/s (Neutec Group Inc.191 masticator; Torrent de l'Estadella, 22 08030 Barcelona, Spain) with distilled water (1:10 w/v). The pH values were recorded by using a pH meter (OrionTM, model 720A, Orion Research Inc., Beverly, MA, USA) into the stirred slurry.

Preparation of Bacterial Suspension

A loopful of frozen stock (at -70 °C) of Listeria monocytogenes (strain V7, serotype $\frac{1}{2}$ a; FDA isolate) obtained from Center for Food Safety laboratory (Favetteville, AR) was transferred to brain heart infusion (BHI) broth (10 mL) and incubated (New Brunswick Scientific agitating incubator at 200 rpm; Edison, NJ, USA) at 37 °C for 24 h. About 10 µL of the culture was inoculated into 10 mL of fresh BHI, and incubated at 37 °C for 18 h. The incubated cultures after 18 h were centrifuged (J2-21 Centrifuge, Beckman, Fullerton, CA, USA) at 37 °C for 10 min to obtain the supernatant and the culture pellets. The pellets were washed twice with phosphate buffer saline (PBS) and re-suspended in the volume of PBS that was equal to the original volume of BHI in the culture. Serial dilutions of the bacterial suspension were made to obtain approximately 10⁵ cfu/mL for surface inoculation of the hotdog samples.

Surface Inoculation of the Hotdog Samples

Hotdogs were sliced into cubes $(1.5 - 2 \text{ g}; 1 \text{ cm}^3)$ and used for surface inoculation as described by [20]. We used

cubed hotdog samples (for better handling and control over experimental conditions under laboratory setting) as model system instead of full hotdog for surface inoculation as demonstrated by previous studies [21]. For surface inoculation, hotdog samples were dipped into the *L. monocytogenes* cultures (~10⁵ cfu/mL) for 1 min and air dried for 20 min under the laminar hood that enabled the bacterial cells to attach to the surface of hotdog samples (to achieve ~4.6 log cfu/g). A total of 588 hotdog samples [2 meat types (chicken and turkey) X 2 fat levels (low and high) X 7 treatments including control X 7 sampling days X 3 replications] were inoculated. After drying, each inoculated hotdog sample was transferred to a sterile whirlpak bag (7.5 x 12.5 cm²; 2.25 mil thickness and 276.CC/100 Sq Inch), vacuum packed (VacMaster-VP 215, Portland, OR, USA) and stored at 4 °C for 28 days.

Bacterial Enumeration

Three hotdog samples per treatment were observed on each sampling day 0, 4, 8, 12, 16, 21, and 28 days to determine the inhibitory activity of PL and SD combination against the growth of *L. monocytogenes*. Phosphate buffer saline (PBS at pH 7.0) was added to the stomacher bags to make a 10 fold dilution and stomached for 120 s to form a homogenate. Stomached samples were serially diluted with PBS, spread plated onto oxford agar with selective supplement, and incubated at 37 °C for 48 h for colony enumeration.

Experimental Design and Statistical Analysis

The experiment design was a split plot where the whole plot portion was completely randomized with [two meats (chicken and turkey) X two fats (high and low fat) X seven PL-SD combinations (including control i.e. no PL and SD)] and the split-plot factor was the seven storage times (0, 4, 8, 4)12, 16, 21, and 28 days) with three replications (3 hot dog samples at each sampling time). (Fig. 1). Observations over time (repeated measures) were considered as split-plot component and the rest of the factors (fat, meat, and treatment) as whole-plot component. For each storage time (day), growth inhibitions (log cfu/g) were determined as the difference between mean $\log (cfu/g)$ count of the control and the mean log (cfu/g) count of treatment sample (PL-SD combination) on that particular day. Analysis of variance was performed using PROC MIXED in SAS® version 9.2 (SAS Institute, Cary, NC, USA) and used to determine statistical differences among the main effects and their interactions with a significance level of P < 0.05. Significant differences among the least square means were used (P < 0.05) among the treatments to identify the effective PL-SD combination.



Fig. (1). Schematic diagram of experimental design involving low and high fat chicken and turkey hotdog treatments to determine the effective combination of potassium lactate and sodium diacetate against surface inoculated *Listeria monocytogenes*.

RESULTS AND DISCUSSION

Proximate Analyses, pH and Water Activity of Hotdog Samples

The percent moisture, fat, protein, residual nitrite, and pH of the hotdog samples determined on day zero are presented in Table **1** (**A** & **B**). Protein content was significantly higher (P < 0.05) in chicken low fat hotdogs when compared to the other hotdog samples (high fat chicken, low and high fat turkey hotdogs) as it was prepared from skinless, boneless chicken breast. Furthermore, there were significant differences (P < 0.05) between chicken and turkey hotdogs in residual nitrite content (ppm) with chicken hotdog samples having significantly higher (P < 0.05) nitrite than turkey samples (Table **1A**).

The initial pH of the control samples (no lactates and diacetates) was 6.12 to 6.50 in low and high fat chicken and turkey hotdogs (Table **1B**). Addition of antimicrobials at varying combination levels significantly (P < 0.05) reduced the pH. Hotdog samples formulated with higher levels of PL (2.0% or 3.0%) and SD (0.15% or 0.2%) reduced the pH in all meat and fat treatments (Table **1B**). Addition of PL and SD at varying levels in hotdog formulations did not signifi-

cantly (P > 0.05) reduce the water activity (a_w) (data not shown) when compared to control samples suggesting that these samples provided favourable conditions for the growth of *L. monocytogenes* [22].

Effect of PL and SD Combinations Against the Growth of *Listeria monocytogenes*:

In Low and High Fat Chicken Hotdog Samples

Growth of *L. monocytogenes* on surface inoculated low and high fat chicken hotdog samples are presented in Fig. (**2 A**, **B**). In low and high fat chicken hotdogs, control samples that did not have PL and SD combination supported rapid growth of *L. monocytogenes* until spoilage (\geq 9.0 log cfu/g by 28 days of storage). However, incorporation of PL and SD at various combination levels achieved variable levels of growth inhibition of *L. monocytogenes* (Fig. **2 A**, **B**). Treatments with 1.0% PL and 0.15% SD and 1.0% PL and 0.20% did not effectively inhibit the growth of *L. monocytogenes* due to low concentration of antimicrobials (lactates and diacetates) in the hotdog samples. Hotdogs formulated with higher levels of PL-SD combinations (2 - 3% PL and 0.15 - 0.20% SD) demonstrated more growth inhibition compared to control. In chicken control samples, growth of

 Table 1A.
 Percent Moisture, Protein, Fat and Residual Nitrite Determined in Low and High Fat Chicken and Turkey Hotdogs (No Lactate and Diacetate).

Treatment	Fot	Moisture	Protoin $(9/)$	$\mathbf{Fat}(9/)$	Residual nitrite (ppm)
Meat	Fat	(%)	riotem (78)	Fat (70)	
Chicken Low		71.26 ^a	20.12ª	5.12 ^b	0.99ª
High		60.67 ^b	15.91 ^b	20.51 ^a	1.17 ^a
Turkey Low		69.24 ^a	15.56 ^b	5.08 ^b	0.59 ^b
High		58.24 ^b	14.57 ^b	20.34 ^a	0.47 ^b

Values are the average of three replications.

Means followed by same superscripts in the same column are not significantly different (P > 0.05). ^adenote highest number, ^bdenote 2nd highest number, ^cdenote 3rd highest number, and ^ddenote lowest number.

Table 1B. pH Values Determined in Low and High Fat Chicken and Turkey Hotdog Samples

		Chicken		Turkey		
Treatment	PL %	SD%	Low fat	High fat	Low fat	High fat
С	0.00	0.00	6.12ª	6.25ª	6.50 ^a	6.40 ^a
T1	1.00	0.15	6.09 ^b	6.21ª	6.45ª	6.36 ^a
Т2	1.00	0.20	6.05 ^b	6.00 ^b	6.30°	6.25 ^{bc}
Т3	2.00	0.15	6.03°	6.03 ^b	6.36 ^b	6.20 ^c
T4	2.00	0.20	5.94 ^d	5.93°	6.26 ^d	6.12 ^d
Т5	3.00	0.15	6.01°	6.02 ^b	6.27 ^{cd}	6.26 ^b
Т6	3.00	0.20	5.94 ^d	5.93°	6.20 ^e	6.15 ^d

Values are the average of three replications.

Means followed by same superscripts in the same column are not significantly different (P > 0.05). ^adenote highest number, ^bdenote 2nd highest number, ^cdenote 3rd highest number, and ^ddenote lowest number.

Treatments - (T; PL, SD): [(Control; 0, 0), (T1; 1.0, 0.10), (T2; 1.0, 0.20), (T3; 2.0, 0.15), (T4; 2.0, 0.20), (T5; 3.0, 0.15) and (T6; 3.0, 0.20)





Data are mean log numbers (cfu/g) from three replications observed on 0, 4, 8, 12, 16, 21, and 28 days of storage. Treatments - (T; PL%, SD%): [(Control; 0, 0), (T1; 1, 0.15), (T2; 1, 0.2), (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)]. Minimum level of detection is 100 cfu/g.

L. monocytogenes was higher in high fat samples than low fat samples on all observation days. Considering the growth inhibition of L. monocytogenes, there were no significant differences (P > 0.05) between low and high fat samples until 4th day of storage (4 °C). However, significant differences (P < 0.05) in growth inhibition between low and high fat samples were apparent from 8th day with higher growth inhibition values (log cfu/g) in low fat samples. The most effective treatment in low and high fat chicken hotdog samples was the combination of 3.0% PL and 0.15% SD with 3.4 log cfu/g and 2.4 log cfu/g growth inhibitions on the 16th and 21st days of storage, respectively. Higher growth inhibitions in low fat hotdog samples can be attributed to increased action of these antimicrobials in the water phase thus exhibiting the inhibitory activities against the growth of L. monocytogenes. These results were not consistent with findings of Hu and Shelef [15] that inhibitory activities of lactates increased with fat content in the beaker sausages. Furthermore, previous studies conducted to determine the effect of fat content on the growth behaviour of *L. monocytogenes* in dairy foods such as cheese and yogurt reported that fat content had no effect on the growth pattern of *L. monocytogenes* [23]. These differences were may be due to the variation in the sensitivity of the strain used in dairy (Scott A vs V 7), product characteristics (pH, moisture, water activity), and model system matrix.

In Low and High Fat Turkey Hotdog Samples

Growth of *L. monocytogenes* in low and high fat turkey control hotdog samples over 28 days of storage at 4 °C are presented in Fig. (**3 A, B**). In low and high fat turkey samples, the initial (day 0) bacterial count was approximately 4.6



Fig. (3). (A & B). Effect of Potassium Lactate (PL) and Sodium Diacetate (SD) combination against *Listeria monocytogenes* in (A) low fat and (B) high fat turkey hotdogs vacuum packed and stored at 4 °C.

Data are mean log numbers (cfu/g) from three replications observed on 0, 4, 8, 12, 16, 21, and 28 days of storage.

Treatments - (T; PL%, SD%): [(Control; 0, 0), (T1; 1, 0.15), (T2; 1, 0.2), (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)]Minimum level of detection is 100 cfu/g.

log cfu/g, while the maximum growth observed until spoilage by 28 days of storage at 4 °C was 8.1 log cfu/g and 6.2 log cfu/g, respectively. Interestingly, growth of L. monocytogenes in turkey high fat samples was low over 28 days of storage at 4 °C. For example, in high fat control samples, L. monocytogenes population has grown from 4.7 log cfu/g to 6.4 log cfu/g by 28 days. Furthermore, in turkey high fat samples, treatments formulated with 2.0% PL and 0.15% SD or higher [(T; PL %, SD %): (T3; 2, 0.15), (T4; 2, 0.2), (T5; 3, 0.15), and (T6; 3, 0.2)] demonstrated listeriostatic activity until 16 days of storage. However, this growth behaviour in high fat system was not consistent in the low fat samples. Considering the growth inhibition of L. monocytogenes, there were significant differences (P < 0.05) between low and high fat samples on all sampling days of storage. Growth inhibitions were higher (P < 0.05) in low fat turkey samples until 21st day and thereafter, high fat samples exhibited higher growth inhibitions on 28th day. The most effective treatment in low and high fat turkey hotdog samples was the combination of 3.0% PL and 0.20% SD with 3.2 log cfu/g and 1.6 log cfu/g growth inhibition on 16^{th} and 12^{th} day of storage, respectively (Fig. **3 A**, **B**).

Pathogen survivors following antimicrobial intervention (stressor: lactate and diacetate combination) stimulate their protective mechanisms [24]. However, inclusion of additional hurdles (storage temperature and vacuum packaging) that contribute to different mechanism(s) may be more effective in pathogen inhibition. Inhibition of L. monocytogenes in this study would be due to several factors. A combination of factors such as lactate and diacetate combinations, pH, vacuum packaging (reduce the redox-potential), and storage temperature may have resulted in the varying antimicrobial activity of the treatments; suggesting that a multiple hurdle approach can inhibit the growth of L. monocytogenes during contamination [25]. Potassium lactate and sodium diacetate are widely used in combination in various meat and poultry products to enhance food safety and extend the shelf life [11,13]. Findings as discussed in previous sections were consistent with previous research findings that demonstrated varying levels of inhibition with PL-SD combination levels

between 2 to 3% PL and 0.15 to 0.2% SD in various meat models [26,27].

These results also suggested that fat level has significant effect on the antimicrobial activity of the lactates and diacetates against the growth of L. monocytogenes. It is likely that high fat samples with lower growth inhibition could be due to the protective influence of fat (less water, pathogen cell protection) from the interaction of lactates and diacetates with the pathogen. This protective effect could either be due to physical protection of the bacterial cell or due to other types of interaction between fat in the meat and bacterial cell wall lipids [23]. In addition, in bi-phasic foods such as hotdogs (oil-in-water emulsion), lipid component of the food is vital in controlling the concentration of undissociated antimicrobial compounds in the aqueous phase. This is due to the lipophilic nature of the undisocciated organic acids; partitioning between aqueous and lipid compoenents of foods, and thus ultimately decreasing the concentration of undissociated organic acids (lactates and diacetates) in the aqueous phase [17].

Differences in the effective combinations of PL-SD determined in this study between meat and fat types against *L. monocytogenes* can also be attributed to the chemical composition and food structure, and thus may affect the bacterial attachment and growth of the pathogen [28]. Factors such as meat composition, type and level of unsaturated fatty acids, present in chicken and turkey meat may contribute to variances, as the unsaturated fatty acids are known to inhibit gram-positive foodborne pathogens such as *L. monocytogenes* [29,30].

CONCLUSIONS

Results of this study showed that, PL and SD when incorporated in hotdog formulations provided significant inhibition of *L. monocytogenes* growth at 4 °C. Combination of 3.0 % PL and 0.15% or 0.20% SD demonstrated effective growth inhibitions against *L. monocytogenes* than the other treatments. Fat content had a significant effect on the growth inhibition of *L. monocytogenes* as indicated by high fat samples having lower growth inhibition in chicken and turkey meat system. Current usage levels of PL ($\leq 2\%$) and diacetates combination in commercial hotdog formulations may not provide effective inhibition of *L. monocytogenes* when the product becomes contaminated. Therefore, other hurdle technologies in addition to using effective concentration levels of chemical antimicrobials need investigation to obtain minimum detectable levels of pathogens.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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