Supplementary S3
9 Days of Shelf-Life Extension in Poultry Frankfurters With Acetic Acid-Based Antimicrobial Solutions

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I. INTRODUCTION

The incidence of *Listeria monocytogenes* in processed meat products ranged from 1.8-4.1% [1]. Traditionally lactic, acetic acid, or their sodium salts were regarded as primary meat safety solutions. However, market trends of low sodium options have pushed the industry to look for alternate solutions. Highly effective potassium-based acetic acid salts (Provian® K) are a very effective solution to meet this need [2]. The objective of this research was to investigate the effect of Provian® K on the growth of spoilage microorganisms (*Lactobacillus sakei*) and the control of *L. monocytogenes* on poultry frankfurters.

II. MATERIALS AND METHODS

A total of 2000 poultry frankfurters for thirteen different treatment formulations (0.25-0.75% Provian® K, 1.25%-3.5% potassium lactate/sodium diacetate blend, and control (no antimicrobials) were inoculated in pairs with *L. monocytogenes* (serotype 1/2a, 1/2b, 4a and 4b) or *L. sakei*. Uninoculated samples were used to detect the level of natural contamination. Following bacterial attachment, the poultry frankfurters were vacuum packaged and stored at 4°C and sampled for up to 120 days. At each sampling, poultry frankfurters were homogenized, stomached, and plated a onto selective medium and incubated at 35°C for 48 h. Failure of anti-listerial capacity was assessed at 2 log CFU/g outgrowth and spoilage threshold was considered as 6 log CFU/g and treatment performances were compared using one-way ANOVA. Data was fitted to the modified Gompertz model to calculate growth rate (μmax; log/day), lag time (day) and estimate day of spoilage. Statistical analysis and model building were carried out using JMP Pro version 15.1.0 (SAS Institute Inc., NC, US), with significance set at $p<0.05$. Data depicted in the tables is the mean ± standard deviation. Values indicated by different letters are significantly different ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

An inoculation level of ca. 3 log CFU/g for all bacteria strains was achieved on day 0 for all the treatments. For *L. monocytogenes*, the control treatment exhibited the fastest outgrowth >2 log CFU/g by 17 days of storage at 4°C. The 0.75% Provian® K treatment significantly *($p<0.05$)* controlled *L. monocytogenes* outgrowth (<2 log CFU/g) for 135 days of storage. Figures S1 and S2 represent the populations of *L. monocytogenes* and *L. sakei* in poultry frankfurters stored at 4 ˚C, respectively. Likewise, for spoilage bacteria, 0.75% Provian® K showed optimal performance across both temperatures of 4°C (Table S1). For *L. sakei*, controls spoiled at 24 days, while 0.75% Provian® K provided significant *($p \leq 0.0032$)* extension of 11 days at 4°C, respectively. Uninoculated samples stored at 4°C did not spoil (<2 log CFU/g) within the 150 days of sampling, ensuring low background loads of bacteria. The data depicted is the mean ± standard deviation. Values indicated by different letters are significantly different (*$p \leq 0.05$*).

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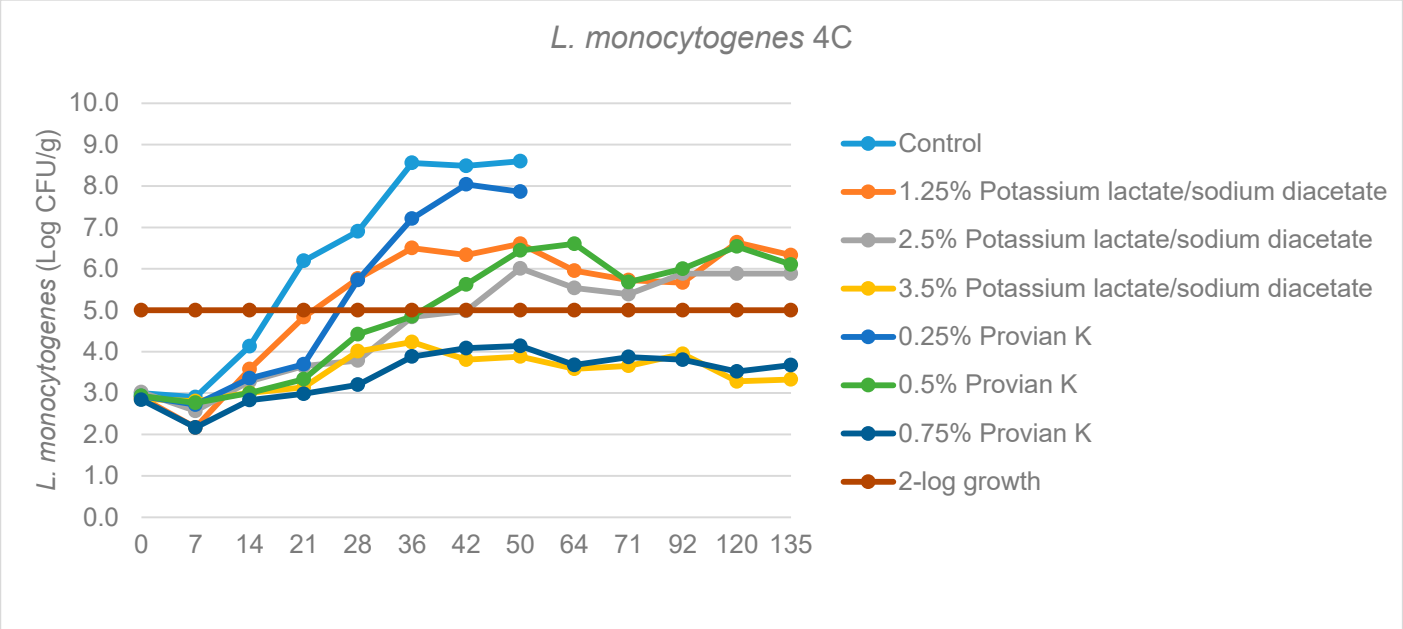


Figure S1. Populations of *L. monocytogenes* in poultry frankfurters at 4 ˚C.

Table S1. Time (days) to *Listeria monocytogenes* 2 log outgrowth at 4 ˚C.

Treatment	Days to 2 Logs *L. monocytogenes*
Positive Control, no antimicrobial	**17±0.05^{A}**
1.25% Potassium Lactate/Sodium Diacetate	**21±0.38^{B}**
2.5% Potassium Lactate/Sodium Diacetate	**40±0.32^{C}**
3.5% Potassium Lactate/Sodium Diacetate	**135^{D}**
0.25% Provian K	**26±0.19^{E}**
0.5% Provian K	**38±0.17^{C}**
0.75% Provian K	**135^{D}**

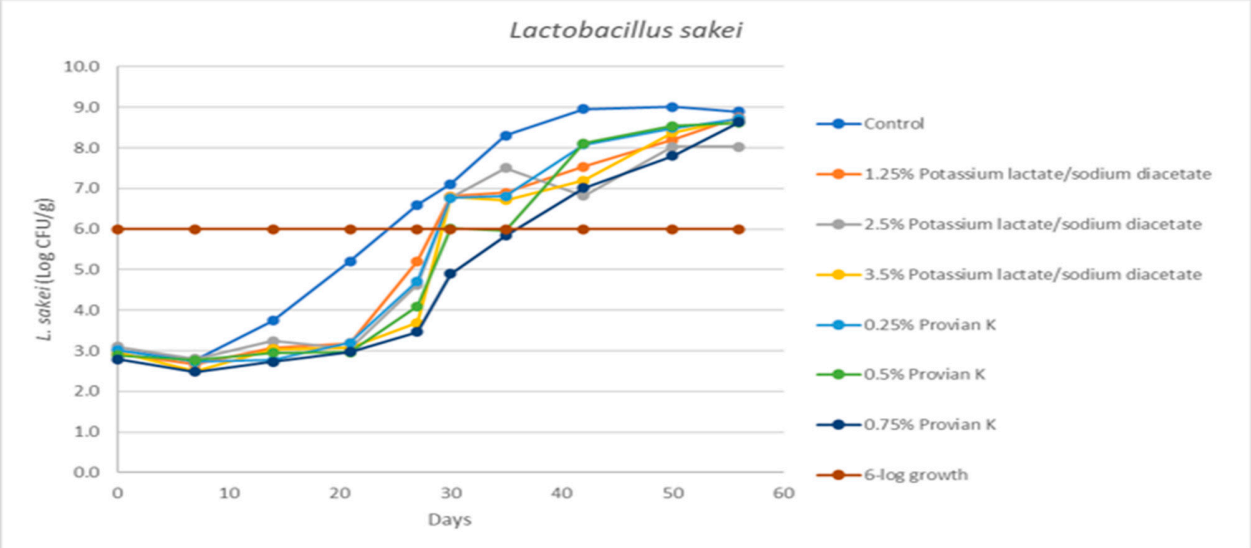


Figure S2. Populations of *L. sakei* in poultry frankfurters at 4 ˚C.

Table S2. Time (days) to end of shelf life (lactic acid bacteria at 6 log CFU/g) at 4˚C.

Treatment	Days to 6 Logs *L. sakei*
Positive Control, no antimicrobial	**24±0.18^{A}**
1.25% Potassium Lactate/Sodium Diacetate	**29±0.18^{B}**
2.5% Potassium Lactate/Sodium Diacetate	**33±0.32^{C}**
3.5% Potassium Lactate/Sodium Diacetate	**33±0.02^{D}**
0.25% Provian K	**29±0.11^{B}**
0.5% Provian K	**32±0.17^{C}**
0.75% Provian K	**35±0.03^{E}**

The data depicted is the mean ± standard deviation. Values indicated by different letters are significantly different *(p≤0.05)*.

IV. CONCLUSION

Provian® K exhibited superior antimicrobial efficacy against both *L. monocytogenes* and spoilage microorganism in processed meat at a 5 times lower use level compared to potassium lactate-based preservatives, providing meat processors with very cost-efficient antimicrobial solutions.

References

1. Seman, D.L., Borger, A.C., Meyer, J.D., Hall, P.A., Milkowski, A.L. (2002). Modeling the growth of *Listeria monocytogenes* in cured ready-to-eat processed meat products by manipulation of sodium chloride, sodium diacetate, potassium lactate, and product moisture content. Journal of Food Protection 65: 651–658.
2. Heintz, E., Van Lent, H. J., Vega, L., Glass, K., (2018). The inhibitory effect of a sodium free powder preservative on the growth of *Listeria monocytogenes* and Lactic acid bacteria in turkey ham applications. Meat and Muscle Biology 1:2.

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Supplementary S4

Shelf-Life Extension of Fresh Chicken Data

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I.INTRODUCTION

Natural vinegar-based solutions have been known to possess antimicrobial properties which could potentially extend the shelf life of poultry meat (Heintz et al., 2019; Heintz et al., 2018). The present study was conducted to evaluate the effect of adding different combinations of vinegar on the shelf life of chicken tenders.

II. MATERIALS AND METHODS

Fresh, chilled chicken tenderloins were procured in bulk from a commercial poultry processing facility on the day of the experiment. Chicken tenders were marinated in different concentrations of IsoAge 220 L using vacuum tumbling and immersion marination techniques. The marinade mixtures contained chilled water, salt, and IsoAge 220 L at a concentration of 1.00 or 2.00% to achieve a marinade pick-up percentage of 10%. A brine solution containing only salt and water served as the control treatment. Chicken tenders were subjected to vacuum tumbling and immersion marination technique using each antimicrobial blend or control treatment at 8-10 rpm for 30 min at 4˚C using a tumbling machine. Post-marination the chicken tenders were vacuum packaged and stored at 4°C for up to 35 days. On each sampling day I.e. day 0, 2, 4, 6, 8, 10,12, 21, 27, and 34, two samples/treatments, were removed from the 4˚C incubator, rinsed using Buffered Peptone Water (BPW) and plated onto Plate Count and de Mann, Rogosa, and Sharpe agar for the enumeration of Aerobic Plate Count (APC) and Lactic Acid Bacteria respectively. The spoilage threshold and maximum population density at the end of stationary phase were considered as 6 log CFU/g, respectively. Data generated were used for primary modeling using a modified Gompertz equation to the calculate maximum growth rate (μmax; log/day) and days to reach the spoilage threshold for each treatment. Differences among the treatments were determined using one-way ANOVA. Statistical analysis and model building were performed in JMP Pro version 15.1.0 (SAS Institute Inc., NC, US), with significance set at $p \leq 0.05$. D. Table 1 shows the days to spoilage (6 log CFU/mL) as mean ± standard deviation with different letters indicating significant differences between the treatments.

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III. RESULTS AND DISCUSSION

Overall, marinating the chicken tenders via vacuum tumbling and immersion marination technique in IsoAge 220L at a concentration of 2.0% proved to be more effective than the control and IsoAge 220 L at 1.0% concentration in controlling the growth of APC and LAB in chicken tenders. Specifically, IsoAge 220L at 2.0% extended the shelf life of chicken tenders by 26 days in terms of both APC and LAB, followed by IsoAge 220L at 1.00% concentration which resulted in the shelf-life extension of 14-15 days as compared to control treatment which reached spoilage threshold (6 log CFU/mL) between 6-7 days of refrigerated storage.

Table S1. Time (days) to end of shelf life (6 log CFU/mL) at 4˚C.

Treatments	Days to 6 logs CFU/mL	
	Aerobic Plate Count (APC)	Lactic Acid Bacteria (LAB)
Control (Brine solution)	6.45 ± 0.35^{A}	6.22 ± 0.02^{A}
1.00% IsoAge 220 L	20.69 ± 2.56^{B}	21.74 ± 0.73^{B}
2.00% IsoAge 220 L	32.77 ± 2.47^{C}	32.70 ± 1.26^{C}

$^{A-C}$Means with different letters represent significant differences between the treatments at p≤0.05

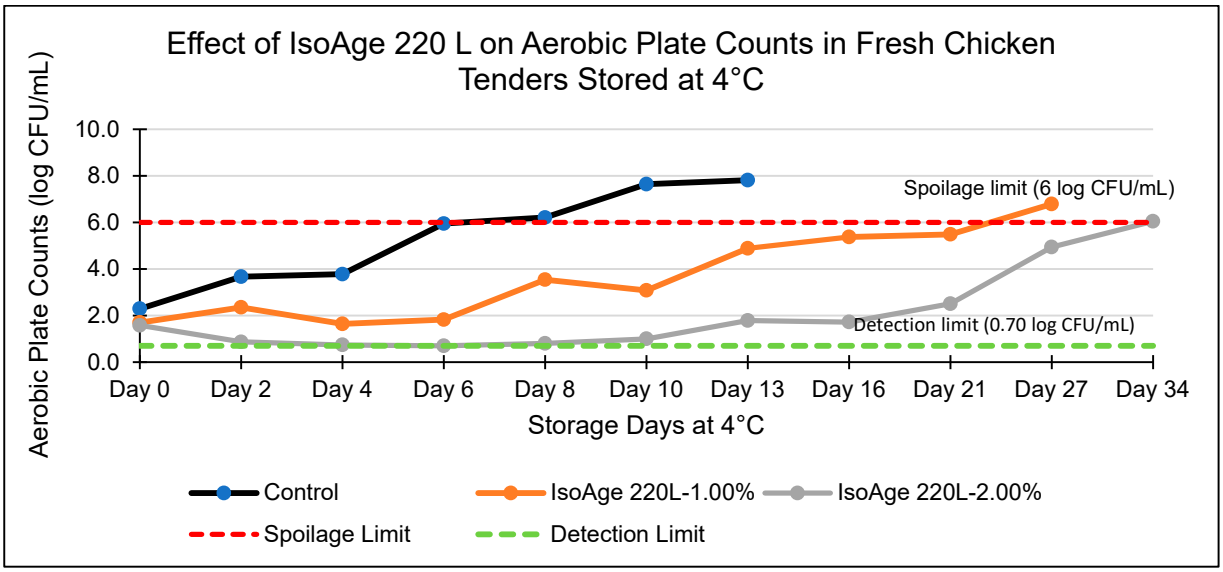


Figure S1. Time (days) to end of shelf life (6 log CFU/mL) at 4˚C.

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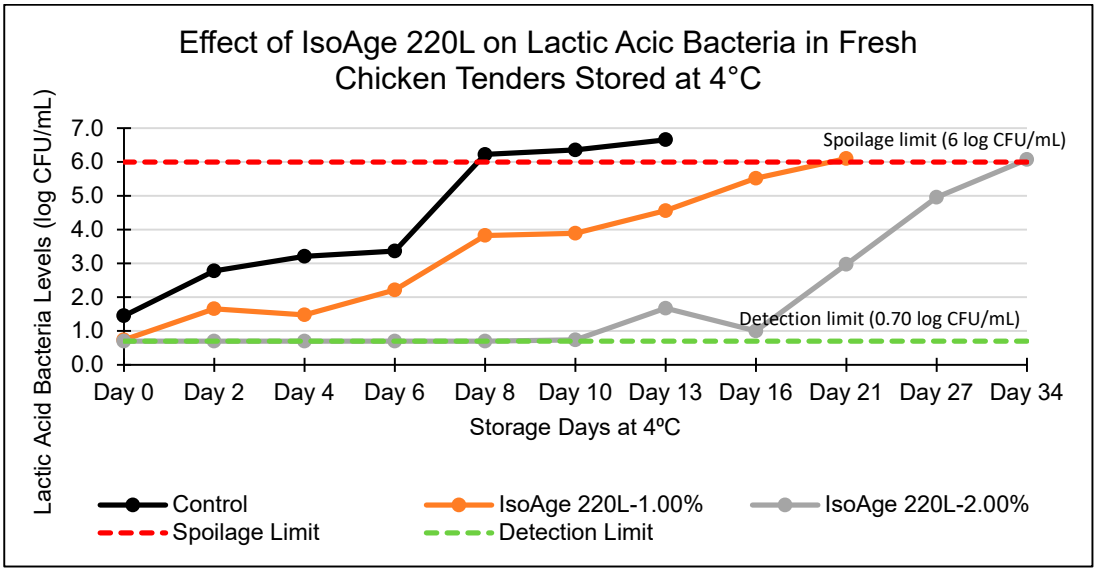


Figure S2. Time (days) to end of shelf life (6 log CFU/mL) at 4˚C.

IV. CONCLUSION

The study confirms the effectiveness of IsoAge 220L as an antimicrobial agent when used as a marinade to combat spoilage microorganisms in poultry meat. This substantiates its potential to extend the shelf life of fresh poultry meat, thereby could eventually aid in reducing food waste.

References

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Heintz, E., Vega, L., & Jahr, G. (2018). Inhibition of *Listeria Monocytogenes* and spoilage bacteria on cured ready-to-eat meats by sodium-free and clean-label antimicrobial ingredients. *Meat and Muscle Biology*, *2*(2).

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