Inhibition of *Listeria monocytogenes* in Deli-style Turkey using Hop Acid Extracts with or without Potassium Acetate and Potassium diacetate

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Abstract –This study was conducted to evaluate the antilisterial activity of hop α- and β-acids with/without potassium acetate and potassium diacetate (PAPD) in deli-style turkey. Deli-meats were traditionally manufactured with/without 5 ppm hop extracts, 0.5% PAPD, or their combinations, which were then inoculated with *L. monocytogenes* (LM) (2-3 log CFU/g) prior to storage in vacuum-package for 60 days or in aerobic-package for 10 days at 4 or 7°C. Deli-style turkey formulated with potassium lactate and sodium diacetate (PLSD), PAPD, β-acid/PAPD, or α-acid/PAPD showed a listeristatic effect for 60 days at 4°C but not at 7°C after 15 days of storage, whereas no inhibition was seen for control, α- and β-acid. In the deli-style turkey, sliced after 30 days of storage and then stored for 10 days in aerobic package, PLSD, PAPD, α-acid/PAPD, and β-acid/PAPD also showed a listeristatic effect at 4 and 7 °C. However, control and α- and β-acid allowed the pathogen to grow by 1.0 and 2.0 log CFU/g at 4 and 7 °C, respectively. Similar results were observed in the deli-style turkey that was sliced after 60 days of storage and then stored for 10 days in aerobic package at 4°C and 7 °C. These results indicated that the combination of hop/PAPD effectively inhibited *Listeria* growth but the single addition of hop acids at 5 ppm failed to inhibit.

Key Words – Hops, Organic acid, *Listeria monocytogenes*, Ready-to-eat product.

I. INTRODUCTION

Outbreaks of listeriosis are frequently related to ready-to-eat (RTE) meat products [5]. According to U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) [13], the delivery time of RTE meats from manufacture to retail store is about 10 to 30 days, where the meats are then displayed for 5 to 30 days depending on sale. After purchase, the meats are usually consumed at home within 1 to 10 days [1]. Partially due to the extended time from manufacture to consumption, both deli meats and non-reheated frankfurters are associated with greater risk for listeriosis among 23 food categories [8].

To control *Listeria monocytogenes* (LM) in RTE meat and poultry products, USDA/FSIS issued an interim final rule requiring one of three alternatives: (i) apply both a post-lethality treatment and an antimicrobial agent; (ii) apply either a post-lethality treatment or antimicrobial agent; (iii) use of sanitation control measures to prevent recontamination after processing [13].

The use of antimicrobial agents including organic acids can increase the safety of RTE meat products [2,3,4]. Previously, our laboratory reported that potassium acetate and potassium diacetate (PAPD) among nine organic acid mixtures showed the best inhibition of LM in frankfurters, but PAPD provided acid taste in low-sodium frankfurters [10].

Hop acids have long been known for antimicrobial activity against Gram positive bacteria [8]. Recently, Sansawat *et al.* found that the combination of hop acid and PAPD demonstrated synergistic effects on *Listeria* inhibition in liquid media [9]. However, no research has been conducted to evaluate antimicrobial activity of hop or hop/organic acid combinations in processed meat formulation. Therefore, this study was designed to assess antilisterial activity of hop extracts with/without PAPD in deli-style turkey meat in the practical situations at manufacture plants and at deli stores or at home.

II. MATERIALS AND METHODS

Deli-style turkey preparation with/without Listeria inhibitors: Seven deli turkey meats were traditionally manufactured by mixing ground turkey breast with water, spice (salt, phosphate, starch, sugar, sodium nitrite, erythorbate) and various inhibitors as follow: (1) inhibitor-free control (CTR); (2) 2.5 % OptiForm® solution of 56% potassium lactate, 4% sodium diacetate, and 40% water (PLSD); (3) 0.5% powdered mixture of potassium acetate (80%) and potassium diacetate (20%) (PAPD); (4) 5 ppm hop alpha acid (α-acid, containing 67.2% α-acid); (5) 5 ppm α-acid and 0.5% PAPD (α-acid/PAPD); (6) 5 ppm hop beta acid (β-acid, containing 96.0% β-acid); and (7) 5 ppm β-acid and 0.5% PAPD (β-acid/PAPD). The meat batters were then stuffed into fibrous casings and cooked to an internal temperature of 74°C in a smoke-free smokehouse. After cooking and showering, the deli turkey chubs were stored overnight at 2°C and sliced for microbiological analysis.

LM strains and inoculum preparation: The cocktail of six LM strains [Lm-10-s11 (serotype 1/2a, delicatessen isolate), Lm-12-s11 (serotype 1/2b, delicatessen isolate), Lm-12-s8 (serotype 1/2b, delicatessen isolate), R3-031 (serotype 1/2a, food isolate from a hot dog outbreak), N1-227 (serotype 4b, food isolate from a deli meat outbreak), and R2-763 (serotype 4b, food isolate from a deli meat outbreak] was prepared in trypticase soy broth with yeast extract to contain ~1 x 10^8 CFU/mL. The cocktail was then serially diluted in sterile phosphate-buffered saline (PBS) to a level of approximately 10^5 CFU/mL of LM for inoculation.

Deli turkey meat inoculation: Listeria inoculation of deli turkey meat was conducted in two different ways to simulate contamination in the plant during manufacture and at retail delis or at home. For a contamination during manufacture, the deli meats were sliced (~ 1.5 mm thick and 25±1 g weight) with a slicer and spot inoculated at several locations on one side with 0.1 mL to obtain 2-3 log CFU/g. The slices were then placed in a biological safety cabinet for 20 min to allow the inoculum to be absorbed. Four slices were placed in each bag, vacuum packaged, and stored at 4 and 7°C. For a contamination at retail delis or at home, the cooked chubs were first stored at 4°C for 30 and 60 days, and then sliced and inoculated as above. Four slices were placed on a piece of delicatessen paper, aseptically transferred to a zip lock delicatessen, and stored at 4 and 7°C for 10 days.

Microbiological analysis: Turkey slices from the day of manufacture and inoculation were assessed for initial populations of LM, and then tested at 15 day intervals for up to 60 days. Slices prepared after 30 and 60 days of storage were analyzed for the pathogen initially and every 2 days up to 10 days. For each treatment, duplicate 25-g samples were diluted 1:10 in PBS and homogenized in a stomacher for 2 min. Appropriate serial dilutions in PBS were plated on modified Oxford agar (MOX) to enumerate LM after incubation for 48 h at 37°C.

Statistical analysis: All experiments were conducted in triplicates. The data were analyzed using the mixed procedure of SAS software. To better assess the effect of treatment on *Listeria* inhibition, the slope of graph of *Listeria* population during storage for each treatment was calculated. Mean differences of LM population or slope of the graph between treatments were determined using Tukey's Test at a = 0.05 level.

III. RESULTS AND DISCUSSION

The initial populations of LM in every storage were 2.5 ± 0.3 log CFU/g among treatments ($P \geq 0.05$). Deli-style turkey formulated with PLSD, PAPD, β-acid/PAPD, or α-acid/PAPD showed a listeristatic effect for 60 days during storage at 4°C (Fig. 1). However, the addition of α- or β-acid alone allowed the pathogen to grow > 7.0 log CFU/g, which was not significantly different from the control ($P \geq 0.05$). When stored at 7°C for 60 days, PLSD, PAPD, β-acid/PAPD, or α-acid/PAPD allowed LM to grow to 4.1 to 5.6 log CFU/g (Fig. 1).

According to the USDA-FSIS definition for an antimicrobial agent [13], such an antimicrobial agent is a substance that effectively reduces, eliminates, or suppresses microbial growth throughout the shelf life of the products. As a result, the agent should allow no more than 2 logs of growth during the product's shelf life. Therefore, the results of this study suggest that

the combination of 5 ppm α-acid/0.5% PAPD or 5 ppm β-acid/0.5% PAPD could be used under USDA-FSIS alternatives 1 or 2 to inhibit LM in ready-to-eat meat products during the storage at 4 and 7°C.

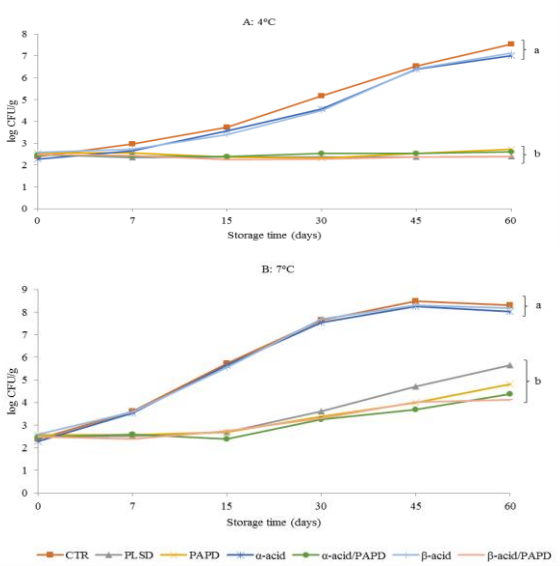

Figure 1. *L. monocytogenes* populations in vacuum-packaged deli-style turkey with various inhibitors during 60 days of storage at 4 (A) and 7°C (B).
a-b Slope of graphs with same letters the same figure were not significantly different ($P \geq 0.05$).

For the deli-style turkey that were sliced after 30 or 60 days of storage and then stored at 4°C for 10 days, *Listeria* populations decreased when PLSD, PAPD, α-acid/PAPD and β-acid/PAPD were added, whereas the pathogen increased by 0.8 log CFU/g in CTR, α-acid and β-acid, with no significant difference ($P \geq 0.05$), regardless of treatment (Fig. 2, 3). During storage at 7°C, listeristatic effects were seen for PLSD, PAPD, α-acid/PAPD and β-acid/PAPD mixtures ($P \geq 0.05$) while both CTR and hop acid alone allowed *Listeria* to grow by 0.3 - 2 log CFU/g (Fig. 2, 3). These results indicated that hop acid formulation at 5 ppm alone was not sufficient to inhibit *Listeria* in deli-meat while the combination of hop acid/PAPD was more effective. The antilisterial activity of hop acids at 5 ppm in deli-turkey meat was different from the results observed in liquid media [9]. These results also support the previous findings that LM was completely inhibited when 3.0 ppm hop β-acid, 1.0% potassium lactate, and 0.25% sodium diacetate were co-added in broth [12]. However, when added singly, a very high concentration of hop β-acid (20,000 ppm) was required to reduce the pathogen by 2.1 log CFU/package [11].

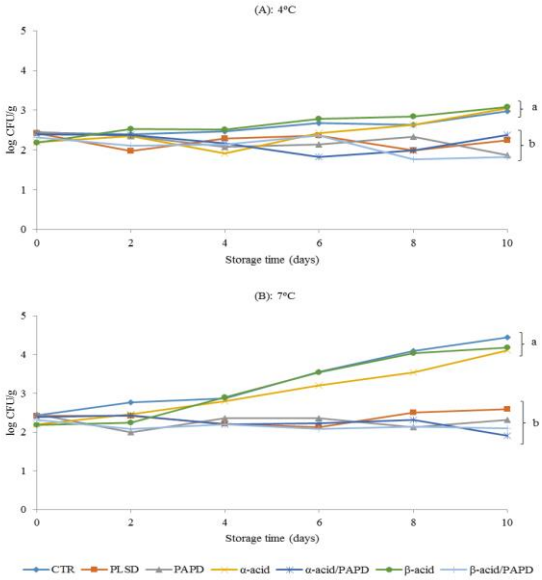

Figure 2. *L. monocytogenes* populations during 10 days of storage at 4 (A) and 7°C (B) in aerobic-packaged deli-style turkey with various inhibitors and sliced after 30 days of storage.
a-b Slope of graphs with same letters the same figure were not significantly different ($P \geq 0.05$).

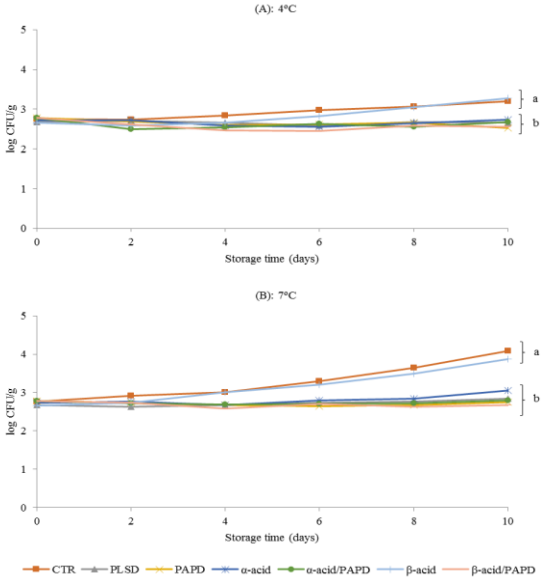

Figure 3. *L. monocytogenes* populations during 10 days of storage at 4 (A) and 7°C (B) in aerobic-packaged deli-style turkey with various inhibitors and sliced after 60 days of storage.
a-b Slope of graphs with same letters the same figure were not significantly different ($P \geq 0.05$).

IV. CONCLUSION

Addition of organic acids in processed meats is one of common intervention strategies to minimize *Listeria* growth. Although antilisterial activity of hop acids has been assessed and well documented using liquid media, their formulation in processed meats has not been studied. Only a few trials were conducted with hops solution sprays or dips for cooked processed meats. This study indicated that hop acids formulation at 5 ppm failed to inhibit *Listeria* growth or did not induce any synergistic effect with 0.5% PAPD in deli turkey meat during storage up to 10 days in aerobic package and 60 days in vacuum package at 4 or 7°C. Based on these findings, the combination of 5 ppm hop and 0.5% PAPD significantly inhibited *Listeria* growth but the single addition of hop acid at 5 ppm appears to be insufficient to be formulated to deli-style turkey.

ACKNOWLEDGEMENTS

The author thanks Kalsec Inc. (Kalamazoo, WI) and Niacet b.v. (Tiel, The Netherland) for funding this project with providing hop extracts and organic acids, respectively.

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