Antilisterial Effects of Hop Alpha and Beta Acids in Turkey Slurry at 7 and 37°C

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Abstract – This study was conducted to investigate antilisterial activities of hop α- and β-acids in turkey slurry. Turkey slurries containing hop α- or β-acids from 0 to 1000 ppm were incubated at 37 °C for 24 h or at 7 °C for 12 days before enumerating *Listeria monocytogenes* populations by plating on modified Oxford agar (MOX) and incubating at 37°C for 48 h. During storage at 37°C for 24 h, *L. monocytogenes* were completely inhibited in the slurries containing α-acid at 750 or 1000 ppm, or β-acid at 1000 ppm, whereas the pathogen grew in the slurries at ≤ 500 ppm, regardless of hop acid types. During storage at 7°C for 12 days, α-acid at ≥ 100 ppm and β-acid at ≥ 500 ppm suppressed the growth of *L. monocytogenes* in turkey slurries for 12 days, with listericidal effect observed in both α-acid and β-acid at 1000 ppm. The α-acid or β-acid at ≤ 50 ppm failed to inhibit *L. monocytogenes* and the growth outcomes were similar to the control slurry ($P \geq 0.05$). Based on these results, the amout of hop acid (α-acid at 100 ppm or β-acid 500 ppm) was required, when they were formulated to meat products and stored at 7°C or less.

Key Words – *Listeria monocytogenes*, Hop acids, Bacterial inhibition, Turkey slurry.

I. INTRODUCTION

Listeria monocytogenes, a psychrotrophic, Gram-positive foodborne pathogen, has been problematic especially in ready-to-eat (RTE) meat products due to frequent re-contamination during post-thermal processing and their ability to grow during refrigerated storage [1,9]. According to the recent report of the Centers for Disease Control and Prevention (CDC), the incidences of infection in 2013 caused by major pathogens including *Listeria* have not been decreased, compared with 2010 – 2012, indicating that there is a gap between the current food safety system and the need for food safety interventions [3].

These concerns prompted efforts to control *L. monocytogenes* in foods and led USDA-FSIS issued the interim final rule regarding the control of *L. monocytogenes* in post-lethality exposed RTE meat and poultry products [13]. According to the *Listeria* Rule, application of the antimicrobial agent to the products is the choice from manufacture's perspective, with the easy and effectiveness to implement. It is also very important to ensure that the antimicrobial agent against *L. monocytogenes* used in food products can control the growth of the pathogen throughout the shelf-life of food products.

Hops (*Humulus lupulus*), mainly used in beer, are known to have antimicrobial properties against Gram-positive bacteria due to the hop acids [2,4,8,12]. Two main hop acids are α-acid and β-acid, which were reported to effectively inhibit *L. monocytogenes* [5, 6,7,11]. However, most studies were conducted in liquid media, while only a few studies have been conducted to evaluate antilisterial effects of hop α- and β-acids using meat slurry to simulate a meat formulation.

Recently, our laboratory found that the formulation of hop α- or β-acid at 5 ppm to deli-tukey meat did not inhibit *listeria* growth nor showed any synergistic effect during storage upon combining with 5% potassium acetate/potassium diacetate (PAPD) although the acid alone at 5 ppm demonstrated both anitislterial and synergistic effects with PAPD in liquid media. Therefore, the purpose of this study was to evaluate antilisterial activity of hop α- and β-acids using the concentration up to 1000 ppm in turkey slurry during the storage for 1 and 12 days at 37 and 7 °C, respectively.

II. MATERIALS AND METHODS

Hop α- (67.2%) and β-acids (96%) were obtained from Kalsec, Inc. (Kalamazoo, WI). A cocktail of *L. monocytogenes* was prepared to contain ~1 x 10^8 CFU/mL with the six strains as follow: 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).

Turkey slurry was prepared by grinding turkey breast through a plate (0.95-cm plate) and mixing the ground turkey (25%) with brine solution (75%), containing 70% water, 2.28% salt, 2.00% sugar, 0.48% phosphate, and 0.24% nitrite for 1 min in a small food chopper. The slurry (100 g) was then pasteurized in each of 250 mL flasks by submerging in a water bath (85°C) while stirring until the internal temperature of 72°C was reached.

Hop α-and β-acids were individually dissolved in 1 mL of 100% ethanol and added to the turkey slurries to achieve the concentrations of 0, 250, 500, 750, and 1000 ppm (w/w) prior to incubation at 37°C or 0, 5, 25, 50, 100, 500, and 1000 ppm (w/w) at 7°C. The *L. monocytogenes* cocktail was then added to each flask to achieve approximately 2.0 – 3.0 log CFU/g, mixed thoroughly, and incubated at 37°C for 24 h or 7°C for 12 days. Samples were taken initially and after 24 h incubation at 37°C, or initially and every 3 days for 12 days at 7°C. Appropriate serial dilutions in sterile phosphate buffered saline (PBS) were plated on modified Oxford agar (MOX) (Difco, BD) and incubated at 37°C for 48 h to enumerate *L. monocytogenes* populations.

All data from triplicate experiments were converted to log CFU/g and presented as mean values. Analysis of variance (ANOVA) was performed using the mixed procedure of SAS software. Statistically significant differences between the treatments were determined using Tukey's Test at α= 0.05.

III. RESULTS AND DISCUSSION

Antilisterial activities of 0 (Control) to 1,000 ppm hop α- and β-acids were evaluated in turkey slurries after incubation at 37°C for 24 h (Table 1).

Table 1 Population of *L. monocytogenes*[1] in turkey slurries containing 0 to 1000 ppm α-acid or β-acid after incubating at 37°C for 24 h.

Treatment	Number of *L. monocytogenes* (log CFU/g)*	
	Time	
	0 h	24 h
α-acid 250 ppm	2.39 ± 0.33[a]	4.38 ± 0.42[cd]
α-acid 500 ppm	2.38 ± 0.34[a]	3.96 ± 0.04[c]
α-acid 750 ppm	2.30 ± 0.30[a]	< 1.00[a]
α-acid 1000 ppm	2.22 ± 0.19[a]	< 1.00[a]
β-acid 250 ppm	2.29 ± 0.47[a]	4.60 ± 0.18[d]
β-acid 500 ppm	2.39 ± 0.32[a]	4.01 ± 0.06[c]
β-acid 750 ppm	2.35 ± 0.33[a]	1.73 ± 0.24[b]
β-acid 1000 ppm	2.35 ± 0.34[a]	< 1.00[a]
0 ppm (Control)	2.40 ± 0.42[a]	8.02 ± 0.31[e]

[1]Means ± standard deviation of $n = 6$ observations for each reading.
[a-e] Mean values with same letters in the same column were not significantly different ($P \geq 0.05$).
* No viable *L. monocytogenes* detection or the counts below minimum detectability of the methodology (10 cells per gram) was marked as < 1.00.

The initial *L. monocytogenes* inoculum level ranged from 2.22 to 2.40 log CFU/g for all treatments, with no significant diffrence at 0 h at 37°C ($P \geq 0.05$). After incubating for 24 h, *Listeria* populations were less than a detection limit (< 10 cells/g) at 750 ppm α-acid and 1,000 ppm β-acids, whereas *Listeria* growth at 500 ppm α- and β-acids was half that of the control (8.02 log CFU/g) (Table 1). Previously, Sansawat *et. al.* [10] found that *Listeria* populations were less than a detection limit (< 10 cells/g) for 50 ppm α-acid in liquid media. Using 25 ppm α-acid and β-acid, *Listeria* populations were reduced to 1/8 and half of control, respectively, in trypticase soy broth with yeast extract (TSBYE) at 37°C for 24 h. The concentration of 25 ppm in liquid media was

about 30 times lower than the requirement for listericidal activity in turkey slurry. Larson *et al.* [6] also reported that *Listeria* growth was completely inhibited in trypticase soy broth containing 10 ppm of hop extract II (41% β- and 12% α-acids) and hop extract III (30% colupulone and 65% β-acids) after 24 h of incubation at 37°C. These findings indicate that the required concentration of hop acid for a listericidal effect in food is much higher than the requirement in liquid media at 37°C.

Antilisterial activities of α- and β-acids at 0, 25, 50, 100, 500, and 1,000 ppm were evaluated in turkey slurries during 12 days of storage at 7°C (Fig. 1).

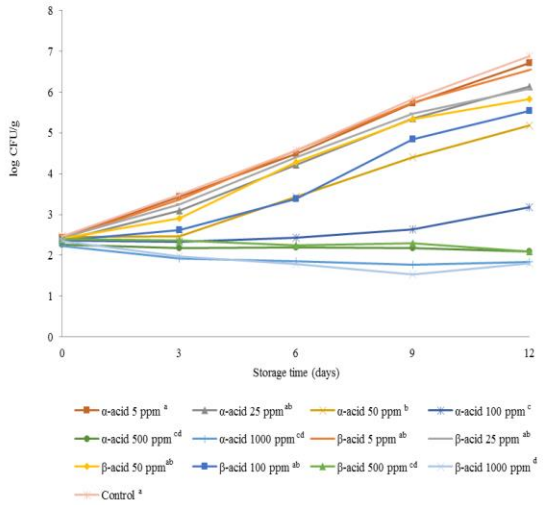

Figure 1. Population of *L. monocytogenes* in turkey slurries containing 0 to 1000 ppm α-acid or β-acid during 12 days of storage at 7°C.

[a-d] Slope of graphs with same letters were not significantly different ($P \geq 0.05$).

Both α- and β-acids were listericidal at > 500 ppm, indicating that the hop concentration required for listericidal effects in turkey slurry is ~100 times higher than in liquid media at 7°C. Although many investigators reported that hop acids at ≤ 10 ppm effectively inhibited the growth of *L. monocytogenes* in liquid media [6,7,11], our study showed that hop acids at < 100 ppm were not effective in turkey slurries at 7°C. In accordance with our results, Larson *et al.* [6] reported that a hop acid extract containing 30% colupulone and 65% β-acids was listericidal at 1,000 ppm in skim milk and 2% milk during 35 days of storage at 4°C, with moderate inhibition and almost no inhibition observed at 100 and 10 ppm, respectively. Again, these findings indicate that the required concentration of hop acids for *Listeria* inhibition in actual foods is not as same as the concentration observed in liquid media.

IV. CONCLUSION

Hop α- and β-acids exhibited antilisterial activity in turkey slurries at the concentrations ≥ 750 ppm during storage at 37°C for 24 h or at the concentrations ≥ 500 ppm at 7°C for 12 days. However, the high concentration of hop acids might not be practical due to the negative hop sensory impacts on turkey. Further studies are required to implement hop acids to meat products with no negative sensory issues. Two potenial solutions could be: 1) combining hop acids with an organic acid at the levels below the threshold for bitter and sour taste, (comma, not period) and 2) encapsulating hop acids to avoid any sequestration by food components during mixing and to maintain antilisterial activity with no negative sensory issue during storage.

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