

PROCESSING AND PRODUCTS

Antilisterial effects of hop alpha and beta acids in turkey slurry at 7 and 37°C

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ABSTRACT Chemical components of hop resins effectively inhibit the growth of *L. monocytogenes* in microbiological culture media. This study was conducted to investigate antilisterial activities of hop α - and β -acid in turkey slurry. Turkey slurries were inoculated with *L. monocytogenes*, formulated with hop α - or β -acid from 0 to 1,000 ppm, and incubated at 37°C for 24 h or at 7°C for 12 days. During storage at 37°C for 24 h, *L. monocytogenes* populations were reduced from 2.40 log CFU/g to non-detectable (<1 log CFU/g) in α -acid at ≥ 750 ppm and β -acid at 1,000 ppm, whereas the control (0 ppm) allowed the pathogen to grow to 8.0 log CFU/g. During storage at 7°C for 12 d, the

slurry treated with α -acid at ≥ 100 ppm and β -acid at ≥ 500 ppm showed listericidal effects, while listericidal effects were observed in the slurries at 1,000 ppm, regardless of hop acid type. Hop α -acid ≤ 50 ppm and β -acid ≤ 100 ppm failed to inhibit *L. monocytogenes*, and the pattern of bacterial growth was similar to that of control with no significant difference ($P > 0.05$). Based on these results, the concentration of α -acid > 100 ppm or β -acid > 500 ppm is minimally required to inhibit *L. monocytogenes* when turkey batters are formulated with hop acids as a single antilisterial agent prior to cooking and storage at 7°C.

Key words: hop acids, *Listeria monocytogenes*, bacterial inhibition, formulation, turkey slurry

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INTRODUCTION

Listeria monocytogenes, a psychrotrophic Gram-positive foodborne pathogen, is likely destroyed in the process of normal cooking, such as cooking to 160°C (an internal temperature) in 70 min (Zaika et al., 1990). The pathogen, however, has been problematic in many foods, not only meat but also non-meat products due to frequent contaminations before/after cooking and the ability to grow regardless of refrigerated storage or not (Sauders and Wiedmann, 2007; Adam and Moss, 2008; McCollum et al., 2013; Choi et al., 2014; McIntyre et al., 2015; CDC, 2016; Foods Safety News, 2017). Upon infection, *L. monocytogenes* is known to result in a very high fatality compared to other bacteria such as *Salmonella* spp. and pathogenic *Escherichia coli* (EFSA, 2014). Gómez et al. (2015) reported that *L. monocytogenes* was detected in cooked (17.1%), raw-cured (36.8%), and dry-cured/salted (24.3%) meat products in Spain. According to the report of the Centers for Disease Control and Prevention (CDC), the in-

cidence of infection in 2013 caused by major pathogens including *Listeria* was not decreased compared with 2010 to 2012, indicating that there might be a gap between the current food safety system and the safety requirement for reducing incidence of bacterial infections (Crim et al., 2014).

A growing number of consumers want to have food products containing natural antimicrobial ingredients rather than synthetic additives/chemical preservatives, antibiotics, or hormones (Winter and Davis, 2006; Devcich et al., 2007; Sharon Palmer, 2013). Hops (*Humulus lupulus*), mainly used in beer, are known to have antimicrobial properties against Gram-positive bacteria due to hop acids (Teuber and Schmalreck, 1973; Hass and Barsoumian, 1994; Bhattacharya et al., 2003; Sakamoto and Konings, 2003). Both α -acid (humulone) and β -acid (lupulone) are the main hop acids that effectively inhibit *L. monocytogenes* by damaging the bacterial cytoplasmic membrane and interfering with active transport of sugar and amino acids (Millis and Schendel, 1994; Larson et al., 1996; Keuleleire, 2000; Shen and Sofos, 2008; Teuber and Schmalreck, 1973).

Hop β -acid has been approved as generally recognized as safe (GRAS) antimicrobials to be used at

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levels not to exceed 4.4 mg/kg (ppm) in cooked meat or 5.5 mg/kg (ppm) in casings for meat products by the U.S. Food Safety and Inspection Service (USDA/FSIS) (US-FDA GRAS Notice Nr 000063, 2001; USDA/FSIS, 2017). However, most research has been conducted in liquid media, while only a few studies have been carried out with real foods such as cheese and milk (Larson et al., 1996; Sansawat et al., 2016). Therefore, the purpose of this study was to evaluate antilisterial activity of hop α - and β -acids in turkey slurries that were fully cooked and stored at 37°C for 24 h and at 7°C for 12 days. The storage temperatures of 37 and 7°C were chosen for a worst case and a temperature abuse case, respectively.

MATERIALS AND METHODS

Preparation of hop acid and *L. monocytogenes* strains

Hop α - and β -acids were obtained from Kalsec, Inc. (Kalamazoo, WI) with concentrations of 67.2 and 96%, respectively (the remainder is primarily non-characterized resinous material and moisture due to the removal of the solvent to FDA trace limits). Six strains of *L. monocytogenes* were obtained from Dr. Martin Wiedmann (Cornell University, Ithaca, NY): 1) Lm-10-s11 (serotype 1/2a, delicatessen isolate), 2) Lm-12-s11 (serotype 1/2b, delicatessen isolate), 3) Lm-12-s8 (serotype 1/2b, delicatessen isolate), 4) R3-031 (serotype 1/2a, food isolate from a hot dog outbreak), 5) N1-227 (serotype 4b, food isolate from a deli meat outbreak), and 6) R2-763 (serotype 4b, food isolate from a deli meat outbreak). Those strains were used to prepare a cocktail of *L. monocytogenes* to contain $\sim 1 \times 10^8$ CFU/mL.

Each strain that was preserved at -80°C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSB-YE) (Difco, Becton Dickinson, Sparks, MD) and 20% glycerin was streaked on TSB-YE agar, and a single colony was picked to grow in 2 consecutive transfers. The resulting colony was subjected to TSB-YE (24 h/37°C), pelleted by centrifugation at 3,100 \times g for 15 min at 4°C, and then re-suspended in sterile phosphate buffered saline (PBS, pH 7.4). Optical density (O.D.) of each cell suspension was measured at 600 nm and adjusted to the same O.D. value. Following the adjustment, 5 mL of each suspension were added to 3 L of PBS to obtain a 6-strain *L. monocytogenes* cocktail at a concentration of $\sim 1 \times 10^8$ CFU/mL. The target population of *L. monocytogenes* in the inoculum was confirmed by plating appropriate dilutions on TSB-YE agar followed by 22 to 24 h incubation at 37°C.

Turkey slurries preparation with hop acids

Turkey slurry was prepared by grinding turkey breast (*Pectoralis major*) 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. The resulting slurry was chilled to 37°C by placing in ice slurry.

Antilisterial activity of hop extracts at 7 and 37°C

Required amounts of hop α - and β -acids were weighed individually, dissolved in 1 mL of 100% ethanol, and added to the turkey slurries to achieve concentrations of 0, 250, 500, 750, and 1,000 ppm (w/w) prior to incubation at 37°C or 0, 5, 25, 50, 100, 500, and 1,000 ppm (w/w) at 7°C. The *L. monocytogenes* cocktail was then added to each flask to achieve approximately 2.0 to 3.0 log CFU/g, mixed thoroughly, and incubated at 37°C for 24 h or 7°C for 12 days. Samples were taken at 0 and 24 h incubation at 37°C, or initially and after incubation for 3, 6, 9, and 12 d at 7°C. Appropriate serial dilutions in sterile PBS were plated with 1 mL in duplicate on modified Oxford agar (MOX) (Difco, BD) and incubated at 37°C for 48 h to enumerate *L. monocytogenes* populations.

Statistical analysis

All experiments were conducted in triplicate. Data were converted to log CFU/g and presented as mean values. Analysis of variance (ANOVA) was performed using the mixed procedure of SAS software (SAS Institute, 2002). Statistically significant differences between the treatments were determined using Tukey's test at the 5% level of significance.

RESULTS AND DISCUSSION

Antilisterial activities of hop α - and β -acids from 0 to 1,000 ppm were evaluated in turkey slurries after incubation at 37°C for 24 h (Table 1). The initial *L. monocytogenes* inoculum level ranged from 2.22 to 2.40 log CFU/g for all treatments, with no significant difference ($P > 0.05$). After incubating for 24 h, *Listeria* populations were less than a detection limit (< 10 CFU/mL) at 750 ppm α -acid and 1,000 ppm β -acids, whereas *Listeria* growth at 500 ppm α - and β -acids was about half the populations of control (8.02 log CFU/g) (Table 1). Previously, Sansawat et al. (2016) reported that the initial inoculation (5.7 log CFU/mL) of *Listeria* became undetectable (< 10 CFU/mL) at 50 ppm α -acid in liquid media at 37°C for 24 h, and significantly reduced to < 2.0 log CFU at 25 ppm α -acid. The concentration of 25 ppm in liquid media was about 30 times lower than the concentration (750 ppm) for listericidal activity in turkey slurry.

Table 1. *L. monocytogenes* populations¹ (mean ± SD) grown in heat-treated turkey slurries containing different concentrations of either α -acid or β -acid after incubating at 37°C for 24 h.

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

^{a-e}Means with different letters within a column were significantly different ($P < 0.05$).

^{A,B}Means with different letters within a row were significantly different ($P < 0.05$).

*No viable *L. monocytogenes* detection was marked as <1.00.

¹n = 3 measurements per mean in each replication.

Larson et al. (1996) reported that *Listeria* growth was completely inhibited in TSB 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. Larson et al. (1996) also showed that no inhibition was observed in skim milk containing 1 and 10 ppm hop β -acid, whereas 100 and 1,000 ppm of hop β -acids were required for listericidal effects.

Antilisterial activities of α - and β -acids at 0, 25, 50, 100, 500, and 1,000 ppm were evaluated in turkey slurries during 12 d of storage at 7°C (Table 2), which was a slightly abusive temperature suggested by the U.S. National Advisory Committee on Microbial Criteria for Foods to reflect consumer handling conditions (NACMCF, 2005). The initial *L. monocytogenes* populations were 2.23 to 2.47 log CFU/g with no significant

difference ($P < 0.05$). Listeristatic effects were found when the concentrations of hop α - or β -acid were at 500 ppm or higher, indicating that the hop concentration for listeristatic effects in turkey slurry is about 83 times higher than in liquid media at 7°C based on the finding that the hop concentration (6.0 ppm) of minimal inhibitory concentration (Sansawat et al., 2016). Our study showed that hop acids at < 100 ppm were not effective in turkey slurries at 7°C.

Results from the research indicated that the hop acid concentration required for listeristatic or listericidal activity in meats is much higher than in liquid media. The discrepancy between liquid media and meat slurry is expected to be due to: 1) less mobility of hop acids in sticky meat slurry or batter than in liquid media, and 2) sequestration of hop acids potentially by fat and protein in the meat prior to their binding to *Listeria* membranes. It has been reported that the hydrophobicity of hop acids can act positively on food lipids and/or microbial membrane (Schmalreck et al, 1975; Larson et al., 1996), resulting in reduced antimicrobial activity of hop acids in high fat levels.

CONCLUSIONS

Hop α - and β -acids exhibited antilisterial activity in turkey slurries at concentrations ≥ 750 ppm during storage at 37°C for 24 h or at concentrations ≥ 500 ppm at 7°C for 12 days. However, the high concentration of hop acids might not be practical due to the negative sensory attributes on turkey. Hop acids flavor was noticeable at >15 ppm, and objectionable flavor was reported at >50 ppm (Millis and Schedel, 1994). Further studies need to be conducted for the effects of hop acids on sensory attributes to meat products.

Table 2. Growth¹ (mean ± SD) of *L. monocytogenes* (log CFU/g) in heat-treated turkey slurries containing different concentrations of either α -acid or β -acid during 12 d of storage at 7°C.

Treatment	Storage day				
	0	3	6	9	12
Control 0 ppm	2.47 ± 0.36 ^{a,A}	3.48 ± 0.23 ^{d,B}	4.57 ± 0.38 ^{d,C}	5.83 ± 0.41 ^{b,D}	6.88 ± 0.61 ^{b,E}
α -acid 5 ppm	2.43 ± 0.43 ^{a,A}	3.44 ± 0.26 ^{d,A,B}	4.49 ± 0.34 ^{d,B}	5.72 ± 0.49 ^{b,C}	6.71 ± 0.52 ^{b,C}
α -acid 25 ppm	2.37 ± 0.45 ^{a,A}	3.10 ± 0.58 ^{b-d,A,B}	4.21 ± 0.34 ^{d,B,C}	5.35 ± 0.63 ^{b,C,D}	6.14 ± 0.62 ^{b,D}
α -acid 50 ppm	2.45 ± 0.43 ^{a,A}	2.46 ± 0.45 ^{a-d,A}	3.44 ± 0.28 ^{c,d,A,B}	4.41 ± 0.48 ^{b,B,C}	5.19 ± 0.63 ^{b,C}
α -acid 100 ppm	2.37 ± 0.33 ^{a,A}	2.33 ± 0.43 ^{a-d,A}	2.44 ± 0.52 ^{a-c,A}	2.64 ± 0.48 ^{a,A}	3.18 ± 0.70 ^{a,A}
α -acid 500 ppm	2.27 ± 0.30 ^{a,A}	2.18 ± 0.37 ^{a-c,A}	2.20 ± 0.48 ^{a,b,A}	2.18 ± 0.58 ^{a,A}	2.10 ± 0.47 ^{a,A}
α -acid 1000 ppm	2.23 ± 0.44 ^{a,A}	1.92 ± 0.42 ^{a,A}	1.85 ± 0.39 ^{a,A}	1.77 ± 0.46 ^{a,A}	1.83 ± 0.50 ^{a,A}
β -acid 5 ppm	2.41 ± 0.44 ^{a,A}	3.36 ± 0.31 ^{d,A}	4.57 ± 0.38 ^{d,B}	5.75 ± 0.43 ^{b,C}	6.54 ± 0.52 ^{b,C}
β -acid 25 ppm	2.43 ± 0.46 ^{a,A}	3.25 ± 0.32 ^{c,d,A,B}	4.41 ± 0.32 ^{d,B,C}	5.48 ± 0.59 ^{b,C,D}	6.09 ± 0.60 ^{b,D}
β -acid 50 ppm	2.42 ± 0.44 ^{a,A}	2.90 ± 0.37 ^{a-d,A}	4.28 ± 0.29 ^{d,B}	5.33 ± 0.45 ^{b,B,C}	5.83 ± 0.70 ^{b,C}
β -acid 100 ppm	2.36 ± 0.46 ^{a,A}	2.62 ± 0.36 ^{a-d,A}	3.38 ± 0.27 ^{b-d,A}	4.84 ± 0.49 ^{b,B}	5.54 ± 0.73 ^{b,B}
β -acid 500 ppm	2.40 ± 0.43 ^{a,A}	2.37 ± 0.49 ^{a-d,A}	2.24 ± 0.63 ^{a,b,A}	2.29 ± 0.66 ^{a,A}	2.10 ± 0.60 ^{a,A}
β -acid 1000 ppm	2.35 ± 0.42 ^{a,A}	1.97 ± 0.43 ^{a,b,A}	1.79 ± 0.45 ^{a,A}	1.54 ± 0.53 ^{a,A}	1.80 ± 0.71 ^{a,A}

^{a-d}Means with different letters within a column were significantly different ($P < 0.05$).

^{A-E}Means with different letters within a row were significantly different ($P < 0.05$).

¹n = 3 measurements per mean in each replication.

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