PROCESSING AND PRODUCTS

Inhibition of *Listeria monocytogenes* in deli-style Turkey using hop acids, organic acids, and their combinations

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ABSTRACT This study was conducted to evaluate antilisterial activity in deli-style turkey using one of the inhibitors: hop α- or β-acids at 5 ppm, potassium acetate/potassium diacetate (PAPD) at 0.5%, their combinations, potassium lactate/sodium diacetate (PLSD) at 2.5% for positive control, and ethanol at 5 ppm for negative control. Deli-style turkey was formulated and manufactured traditionally. To simulate *Listeria* contamination in processing plants, the deli turkey was sliced in <24 h of production, inoculated with *Listeria monocytogenes* (2 to 3 log CFU/g), and vacuum-stored at 4 or 7°C for 60 d. To simulate *Listeria* contamination in grocery stores, the deli turkey was vacuum-stored for 30 and 60 d prior to slicing, inoculation, and aerobic storage at 4 or 7°C for 10 d. Physicochemical properties of the deli turkey were not significantly different among treatments ($P > 0.05$). Addition of hop acids at 5 ppm did not inhibit *Listeria* in deli meat during 60 d of vacuum-storage at 4 or 7°C, whereas organic acids and hop acids/PAPD significantly inhibited *Listeria* ($P < 0.05$), with the best inhibition observed for hop β-acids/PAPD at 7°C/60 d. During 10 d of aerobic storage at 4°C, hop acid/PAPD, PLSD, or PAPD showed listeristatic effects, whereas hop acids alone allowed *Listeria* to grow by 0.9 CFU/g. During 10 d of aerobic storage at 7°C, hop acid/PAPD, PLSD, and PAPD significantly reduced *Listeria* populations than hop α-acids, β-acids, and no-inhibitor control ($P < 0.05$). These results indicated the combination of hop β-acids/PAPD provides more effective inhibition than any single addition of hop acids and PLSD ($P < 0.05$) at 7°C/60 d in vacuum storage, with intermediate inhibition observed for PAPD and α-acids/PAPD.

Key words: antilisteria, hop acids, organic acids, formulation, deli-style turkey

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INTRODUCTION

Consumers believe that natural ingredients can provide health benefits, whereas artificial additives possess health risks. According to a survey, about 63% of people prefer to having products labeled with "natural" due to no artificial ingredients (Weaver, 2014). In respond to the consumer response, food manufacturers have attempted to produce products with natural and clean labels. FDA has not objected to the use of the term of natural if the food does not contain added color, artificial flavors, or synthetic substances (FDA Basics, 2017).

Hops acids and their extracts are plant-origin ingredients that have long been known for antimicrobial activity against Gram-positive bacteria (Schmalreck and Teuber, 1975; Simpson, 1993; Sakamoto and Konings, 2003). Hop β acids were granted generally-recognized-as-safe status, with the approval level of 4.4 mg/kg (ppm) in cooked meat or 5.5 mg/kg (ppm) in casings for meat products (US-FDA 2001; USDA-FSIS 2008). In liquid media, a minimal inhibitory concentration for *Listeria* was reported to be about 5 to 6 ppm of hop α- or β-acids (Millis and Schendel, 1994; Barney et al., 1995; Sansawat et al., 2016). When frankfurter was dipped in hop β-acid solutions from 0.03 to 0.1%, *Listeria* was reduced by 1.3 to 1.6 log CFU/cm^2 during storage for 48 to 90 d (Shen et al., 2009). An immediate reduction of *Listeria* population by 1.2 to 1.5 log CFU/cm^2 was observed after dipping the slices of commercially cured ham in 0.11% hop β-acid solution (Wang et al., 2016).

However, most previous studies have been conducted in liquid media and/or with a food surface application (Seman et al., 2004; Sean and Sofos, 2008; Sansawat

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et al., 2016; Wang et al., 2016). In 1996, Larson et al. added hop extracts to liquid media and actual foods including cottage cheese (pH 4.5) and whole milk. Results indicated that the growth of *Listeria* was inhibited at 10, 100, and 1,000 ppm of hop extracts in liquid media, cottage cheese, and whole milk, respectively. According to Sansawat et al. (2018), the amount of 750 ppm hop α-acid and 1,000 ppm hop β-acid was required, respectively, to inhibit *Listeria* in turkey slurry during storage at 37°C for 24 h. These results indicate that higher amounts of hop acids are required in food products than liquid media, potentially due to sequestration by hydrophobic fat, protein, and other ingredients in foods (Schmalreck and Teuber, 1975; Larson et al., 1996; Sansawat et al., 2018). The high amounts of hop acids (100 to 1,000 ppm) may not be acceptable due to the bitter taste detectable at the levels $\geq$50 ppm (Millis and Schendel, 1994). In addition, the amount of sodium diacetate or potassium diacetate at $\geq$0.2% provides adverse sensory attributes in ham and sausage containing (Stekelenburg and Kant-Muermans, 2001; Sansawat et al., 2013).

Therefore, this study was conducted to evaluate any synergistic effects on antilisteria in the formulation of deli-style turkey at 5 ppm hop acids and 0.5% organic acids (containing 0.1% potassium diacetate) separately and jointly.

MATERIALS AND METHODS

Deli-style Turkey Preparation with Inhibitors

Turkey breasts and ingredients for deli-style turkey were obtained locally. Hops extracts and potassium acetate and potassium diacetate (**PAPD**) were donated from Kalsec Inc. (Kalamazoo, MI) and Niacet b.v. (Tiel, The Netherlands), respectively. Potassium lactate and sodium diacetate (**PLSD**, OptiForm®) was purchased from PURAC America, Inc (Lincolnshire, IL). Deli-style turkey was processed at the Michigan State University Meat laboratory (East Lansing, MI) using a traditinal turkey formuation: turkey breast (71.84%), water (20.44 to 21.94%), salt (1.68%), phosphate (0.36%), starch (2.50%), sugar (1.44%), sodium nitrite (0.18%), erythorbate (0.0578%), and antilisterial additives (0 to 2.5%). Hop α- and β-acids were dissolved individually in 1 mL of 100% ethanol and added to the turkey batter to achieve a concentraton of 5 ppm (w/w), after mixing with batch water for an even distribution. The concentrations of hop α-acid (humulone) and β-acid (lupulone) were specified by Kalsec Inc. for 67.2 and 96.0%, respectively, and the remainder (or carrier) was primarily non-characterized resinous material including tannins, fats, polymers, hop acid by-products, hydrophobic substances, and moistures due to the removal of the solvent to FDA trace limits.

Seven inhibitors were tested in this study as follows: i) 5 ppm ethanol for a negative control; ii) 2.5% of commercial inhibitor for a positive control—56% potassium lactate, 4% sodium diacetate, and 40% water (PLSD); iii) 0.5% powder of potassium acetate (80%) and potassium diacetate (20%) (PAPD); iv) 5 ppm hop α-acid; v) 5 ppm α-acid and 0.5% PAPD (α-acid/PAPD); vi) 5 ppm hop β-acid; and vii) 5 ppm β-acid and 0.5% PAPD (β-acid/PAPD).

Deli-style turkey batter was prepared by mixing for 8 min, using a traditional formulation with 1 of the 7 inhibitors in a bowl chopper (model K64-Va, Maschinenfabrik Seydelmann KG, Aalen, Germany). The meat batter was then stuffed into fibrous casing (90 mm; Devro-Teepak Inc., Danville, IL) and cooked to an internal temperature of 74°C in a smoke-free smokehouse.

Physicochemical Analysis of Deli-style Turkey

Deli turkey meats were assessed for pH, water activity (a_w), moisture, fat, and cooking yield. For pH, a 5-g sample was homogenized in 25 mL of deionized water and then pH was measured with a pH meter (Accumet AR15, Fisher Scientific, Pittsburgh, PA) equipped with an electrode censor (model 13–620-631, Fisher Scientific, Houston, TX). Water activity (a_w) was determined with an AquaLab meter (Decagon Devices, Pullman, WA). Moisture and fat contents were evaluated according to the AOAC International official methods 950.46B and 991.36, respectively (AOAC, 2005). The cooking yield of deli turkey was measured based on the difference of weight before and after cooking.

Listeria Monocytogenes Strains and Inoculum Preparation

Six strains of *L. monocytogenes* obtained from Dr. Martin Wiedmann, Cornell University, Ithaca, NY were used in this study: 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) (Sansawat et al., 2013, 2018).

Each strain that was preserved at –80°C in Trypticase Soy Broth containing 20% glycerin and 0.6% (w/v) yeast extract (TSB-YE, Difco, Becton Dickinson, Sparks, MD) 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 × g for 15 min at 4°C, and then resuspended in sterile phosphate-buffered saline (**PBS**, pH 7.4). Optical density (**OD**) of each cell suspension was measured at 600 nm and adjusted to the same OD value. Following the adjustment, 5 mL of each suspension was added to 3 L of PBS to obtain a 6-strain *L. monocytogenes* cocktail at a concentration of approximately 1×10^8 CFU/mL. The cocktail was then serially diluted in sterile PBS (pH 7.4) to the level of approximately

10^5 CFU/mL of *L. monocytogenes* for deli meat inoculation. The *L. monocytogenes* population in the inoculum was confirmed by plating appropriate dilutions on trypticase soy agar (Difco) with YE and incubating them for 24 h at 37°C.

Deli-style Turkey Inoculation

Listeria inoculation to deli turkey meats was conducted in 2 different ways to simulate a primary contamination at plants manufacture and a secondary contamination at deli stores or home storage. For plant contamination, the deli meats were sliced (approximately 1.5 mm thickness and 25 ± 1 g weight) using a mechanical delicatessen slicer (model 410, Hobart, Troy, OH) within 1 d of production and spot inoculated for several times on one side to obtain 2 to 3 log CFU/g. These slices were then placed under a biological safety cabinet for 20 min to absorb the inoculum at room temperature. Four slices were placed in vacuum bags (18 × 30 cm; VacMaster, Kansas City, MO), vacuum sealed, and stored at 4 and 7°C. To simulate a deli store or home contamination, the remaining sticks were vacuum-stored for 30 and 60 d at 4°C prior to slicing. Each slice was then inoculated as explained above. Four slices were placed on a delicatessen paper (20 × 27 cm; Brown Paper Goods, Waukegan, IL), aseptically transferred, and aerobic sealed using delicatessen bag (20 × 25 cm; Elkay Plastics) prior to storage at 4 and 7°C for 10 d.

Microbiological Analysis

Populations of *L. monocytogenes* were assessed on the days 0, 7, 15, 30, 45, and 60 in vacuum storage and on the days 0, 2, 4, 6, 8, and 10 in aerobic storage. For *Listeria* analysis, duplicate 25-g samples were diluted 1:10 in PBS and homogenized in a stomacher (NEUTEC Group, Farmingdale, NY) for 2 min. The amount (1 mL) of appropriate serial dilutions in PBS were plated in duplicate on modified Oxford agar (MOX) (Difco, BD) to enumerate *L. monocytogenes* after incubation 48 h at 37°C.

Statistical Analysis

The experiment was conducted in triplicate. The microbiological data were converted into log CFU/mL. Analysis of variance was performed using the mixed procedure of SAS software (SAS Institute, 2002). Mean difference between treatments was determined using Tukey's test at $P < 0.05$ level.

RESULTS AND DISCUSSIONS

Deli-style turkey containing no inhibitor, hop acids, organic acids, and their combination were assessed for pH, a_w, moisture, fat, and cooking yield. No significant differences were found among the turkeys, regardless of inhibitors ($P \geq 0.05$, Table 1). The differences in pH, a_w, and fat were less than 0.05 unit, whereas the differences in moisture and cooking yield were less than 1.3 and 3.1%, respectively. In support of our findings, Sansawat et al. (2013) reported that there was no significant difference on a_w, moisture, fat, and cooking yield in frankfurters when *Listeria* inhibitor of PAPD was formulated. Shen et al. (2009) reported that frankfurter dipping in 0.03 to 0.10% hop β-acid solutions did not change pH and a_w of the product.

In evaluation of deli meat pH from 5 commercial brands at the time of delivery, mid-storage, and last allowable date, Zhang et al. (2012) reported that the pH ranged from 6.2 to 6.4, which is similar to our finding (6.26 to 6.30). These results indicated that addition of common antimicrobial agents in 0.5 to 2.5% did not affect ($P \geq 0.05$) the physicochemical property of deli-style turkey breasts.

In order to simulate *Listeria* contamination at manufacture plants, deli turkey was sliced and spot inoculated within 1 d of production, and the resulting slices were stored at refrigerated (4°C) or temperature-abuse (7°C) conditions (NACMCF, 2005). The spot inoculation resulted in an initial contamination of *L. monocytogenes* from 2.28 to 2.59 log CFU/g in deli-style turkey (Table 2). During storage of the deli turkey in vacuum at 4°C for 60 d, both PLSD and hop β-acid/PAPD showed a listericidal effect with the populations reduction by 0.1 to 0.25 log CFU/g, whereas PAPD and α-acid/PAPD demonstrated a listeristatic effect with an initial reduction by 0.16 log CFU/g, followed by recovery of 0.32 log CFU/g. The single addition of α- or β-acids allowed the pathogen to grow by approximately 4.6 CFU/g, which was not significantly different from no inhibitor control ($P < 0.05$) (Table 2).

When the deli turkey was stored at 7°C for 60 d, β-acid/PAPD showed the best antilisterial activity, 3 inhibitors of α-acid/PAPD, PAPD, and PLPD marked an intermediate inhibition, and hop α- or β-acids resulted in the least inhibition, which is not significantly different from the control ($P > 0.05$) (Table 2).

In *Listeria* inhibition using liquid media, Sansawat et al. (2013) reported that a single additon of hop β-acid at 5 ppm showed a listeristatic effect and the combination of 5 ppm hop β-acid and 0.5% PAPD showed a listericidal effect during 6 d of storage at 7°C. In the case of refrigerated or elevated temperature, Blom et al. (1997) demonstrated that the mixture of 2.5% sodium lactate and 0.25% sodium acetate inhibited growth of *L. monocytogenes* in cooked ham throughout 5 wk at 4°C, but the strength of inhibition was redued to 2 to 3 wk at 9°C.

According to the definition of antimicrobial agent defined by USDA/FSIS (2003), antimicrobial agent is a substance that effectively reduces or eliminates microorganisms or that has effect of suppressing or limiting its growth throughout the shelf life of the products and it should allow no more than 2 logs of growth occurred over the product's shelf life. At the end of 60-d storage, the hop β-acid/PAPD combination

Table 1. Impact of hop acids and PAPD on the physicochemical properties[1] of deli-style turkey.

Inhibitor\parameter[2]	pH	a_w	Moisture (%)	Fat (%)	Cooking yield (%)
CTR	6.30 ± 0.04	0.968 ± 0.007	72.96 ± 0.91	0.97 ± 0.37	85.93 ± 2.42
PLSD	6.30 ± 0.04	0.966 ± 0.005	71.34 ± 1.23	1.01 ± 0.41	87.02 ± 3.48
PAPD	6.27 ± 0.05	0.970 ± 0.003	71.69 ± 1.61	0.97 ± 0.47	85.04 ± 3.33
α-acid	6.30 ± 0.06	0.971 ± 0.005	71.71 ± 0.45	0.97 ± 0.41	83.90 ± 0.92
α-acid/PAPD	6.29 ± 0.04	0.970 ± 0.001	72.31 ± 1.66	0.99 ± 0.44	84.78 ± 0.44
β-acid	6.30 ± 0.06	0.972 ± 0.004	72.94 ± 1.33	0.99 ± 0.38	86.47 ± 0.03
β-acid/PAPD	6.26 ± 0.07	0.972 ± 0.005	72.82 ± 1.09	1.01 ± 0.36	85.63 ± 0.91

[1]Number of observations in each parameter per inhibitor, n = 6 except for cooking yield (n = 4).
[2]CTR: No inhibitor control.
PLSD: 2.5% of potassium lactate (56%)/sodium diacetate (4%)/water (40%).
PAPD: 0.5% of potassium acetate (80%)/potassium diacetate (20%).
α-acid: 5 ppm of hop α-acid.
α-acid/PAPD: 5 ppm α-acid/0.5% PAPD.
β-acid: 5 ppm of hop β-acid.
β-acid/PAPD: 5 ppm β-acid/0.5% PAPD.

Table 2. Population[1] of *L. monocytogenes* on vacuum-packaged deli-style turkey with various inhibitors[2] during 60 d of storage at 4 and 7°C.

	Population of *Listeria monocytogenes* (log CFU/g) on storage day:					
Treatment	Day 0	Day 7	Day 15	Day 30	Day 45	Day 60
Storage at 4 °C						
CTR	2.39 ± 0.36^{a}	2.96 ± 0.11^{a}	3.73 ± 0.37^{b}	5.18 ± 0.13^{b}	6.52 ± 0.38^{b}	7.54 ± 0.13^{b}
PLSD	2.48 ± 0.52^{a}	2.33 ± 0.06^{a}	2.37 ± 0.31^{a}	2.37 ± 0.34^{a}	2.36 ± 0.37^{a}	2.39 ± 0.36^{a}
PAPD	2.56 ± 0.37^{a}	2.55 ± 0.29^{a}	2.37 ± 0.15^{a}	2.32 ± 0.32^{a}	2.54 ± 0.60^{a}	2.73 ± 0.51^{a}
α-acid	2.28 ± 0.55^{a}	2.65 ± 0.18^{a}	3.56 ± 0.43^{b}	4.57 ± 0.14^{b}	6.40 ± 0.42^{b}	7.02 ± 0.48^{b}
α-acid/PAPD	2.46 ± 0.17^{a}	2.42 ± 0.31^{a}	2.39 ± 0.26^{a}	2.54 ± 0.47^{a}	2.54 ± 0.34^{a}	2.61 ± 0.60^{a}
β-acid	2.59 ± 0.27^{a}	2.72 ± 0.12^{a}	3.40 ± 0.22^{b}	4.50 ± 0.43^{b}	6.42 ± 0.43^{b}	7.14 ± 0.18^{b}
β-acid/PAPD	2.49 ± 0.29^{a}	2.43 ± 0.38^{a}	2.24 ± 0.14^{a}	2.27 ± 0.47^{a}	2.37 ± 0.38^{a}	2.39 ± 0.44^{a}
Storage at 7 °C						
CTR	2.39 ± 0.36^{a}	3.60 ± 0.51^{b}	5.73 ± 0.33^{b}	7.65 ± 0.24^{b}	8.48 ± 0.19^{b}	8.29 ± 0.10^{c}
PLSD	2.48 ± 0.52^{a}	2.51 ± 0.36^{a,b}	2.68 ± 0.50^{a}	3.60 ± 0.63^{a}	4.71 ± 0.99^{a}	5.64 ± 1.04^{b}
PAPD	2.56 ± 0.37^{a}	2.55 ± 0.32^{a,b}	2.70 ± 0.25^{a}	3.37 ± 0.50^{a}	3.99 ± 0.77^{a}	4.80 ± 0.39^{a,b}
α-acid	2.28 ± 0.55^{a}	3.54 ± 0.49^{b}	5.65 ± 0.37^{b}	7.54 ± 0.25^{b}	8.26 ± 0.16^{b}	8.03 ± 0.11^{c}
α-acid/PAPD	2.46 ± 0.17^{a}	2.58 ± 0.36^{a,b}	2.38 ± 0.28^{a}	3.25 ± 0.54^{a}	3.69 ± 0.77^{a}	4.37 ± 0.59^{a,b}
β-acid	2.59 ± 0.27^{a}	3.59 ± 0.55^{b}	5.54 ± 0.48^{b}	7.69 ± 0.01^{b}	8.30 ± 0.33^{b}	8.18 ± 0.35^{c}
β-acid/PAPD	2.49 ± 0.29^{a}	2.38 ± 0.11^{a}	2.75 ± 0.02^{a}	3.30 ± 0.19^{a}	4.02 ± 0.41^{a}	4.13 ± 0.78^{a}

[a–c]Means with different letters within a column are significantly different ($P < 0.05$).
[1]Number of observations in each storage per inhibitor, n = 6.
[2]Inhibitors as in Table 1.

showed more robust inhibition than any single additive, especially at the elevated storage temperature (7°C) with no more growth than 2 logs (Table 2).

In order to simulate *Listeria* contamination at retails or home refrigerator, vacuumed deli-style turkeys for 30 d at 4°C were sliced using a mechanical delicatessen slicer and spot inoculated with a 6-strain *L. monocytogenes* cocktail. The surface inoculation yielded *L. monocytogenes* populations of 2.18 to 2.45 log CFU/g prior to aerobic package and storage for 10 d at 4 or 7°C (Table 3).

During 10-d storage at 4°C, 4 treatments (PLSD, PAPD, α-acid/PAPD, and β-acid/PAPD) inhibited *Listeria* growth in deli-style turkeys by 0.01 to 0.58 CFU/g, whereas single hop acids and control promoted *Listeria* growth from 2.18–2.45 log CFU/g to 3.0–3.1 CFU/g (Table 3). Regardless of treatment, however, no significant difference was found on *Listeria* populations during the 10-d storage ($P > 0.05$). During the storage at 7°C, significantly reduced *Listeria* populations ($P > 0.05$) were found in 4 treatments (PAPD, α-acid/PAPD, and β-acid/PAPD, and PLSD) than control, α-acid, and β-acid, with listericidal effect found for PAPD, α-acid/PAPD, and β-acid/PAPD as well as listeristatic effect found for PLSD (Table 3).

Deli-style turkeys that were vacuum stored for 60 d were similarly opened, sliced, and inoculated as before. The surface inoculation yielded *L. monocytogenes* populations of 2.66 to 2.78 log CFU/g prior to aerobic storage for 10 d at 4 and 7°C (Table 4). During 10-d storage at 4°C, a similar inhibition and growth of *Listeria* populations were observed as before at 10-d storage. Four treatments (PLSD, PAPD, α-acid/PAPD, and β-acid/PAPD) inhibited *Listeria* growth by 0.01 to 0.49 CFU/g, whereas control and single hop acids supported *Listeria* growth by 0.54 to 0.90 log CFU/g (Table 3). Again, no significant difference was found on *Listeria* populations, regardless of treatment and inhibitor ($P > 0.05$). During storage at 7°C, the 4 treatments (PLSD, PAPD, α-acid/PAPD, and β-acid/PAPD) suppressed

Table 3. Population[1] of *L. monocytogenes* at 4 and 7°C on aerobic-packaged deli-style turkey with various inhibitors[2] and sliced after 30 d of storage whole sticks.

	Population of *Listeria monocytogenes* (log CFU/g) on storage day:					
Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Storage at 4 °C						
CTR	2.43 ± 0.41[a]	2.39 ± 0.36[a]	2.47 ± 0.21[a]	2.68 ± 0.24[a]	2.63 ± 0.57[a]	2.97 ± 0.43[a]
PLSD	2.42 ± 0.43[a]	1.97 ± 0.85[a]	2.28 ± 0.50[a]	2.36 ± 0.10[a]	1.99 ± 0.85[a]	2.24 ± 0.47[a]
PAPD	2.45 ± 0.30[a]	2.35 ± 0.37[a]	2.08 ± 0.43[a]	2.13 ± 0.38[a]	2.33 ± 0.31[a]	1.87 ± 0.81[a]
α-acid	2.20 ± 0.35[a]	2.35 ± 0.56[a]	1.91 ± 0.81[a]	2.43 ± 0.10[a]	2.63 ± 0.54[a]	3.05 ± 0.16[a]
α-acid/PAPD	2.39 ± 0.45[a]	2.38 ± 0.33[a]	2.16 ± 0.41[a]	1.83 ± 0.76[a]	1.99 ± 0.85[a]	2.38 ± 0.17[a]
β-acid	2.18 ± 0.60[a]	2.52 ± 0.31[a]	2.52 ± 0.45[a]	2.78 ± 0.42[a]	2.84 ± 0.35[a]	3.08 ± 0.07[a]
β-acid/PAPD	2.32 ± 0.58[a]	2.10 ± 0.35[a]	2.13 ± 0.38[a]	2.36 ± 0.10[a]	1.77 ± 0.68[a]	1.83 ± 0.72[a]
Storage at 7 °C						
CTR	2.43 ± 0.41[a]	2.76 ± 0.27[b]	2.87 ± 0.51[a]	3.55 ± 0.41[b]	4.09 ± 0.12[b]	4.44 ± 0.21[c]
PLSD	2.42 ± 0.43[a]	2.41 ± 0.37[a]	2.21 ± 0.45[a]	2.12 ± 0.39[a]	2.50 ± 0.44[a]	2.59 ± 0.36[a,b]
PAPD	2.45 ± 0.30[a]	2.00 ± 0.87[a,b]	2.35 ± 0.16[a]	2.35 ± 0.15[a,b]	2.12 ± 0.39[a]	2.32 ± 0.28[a]
α-acid	2.20 ± 0.35[a]	2.47 ± 0.66[a,b]	2.80 ± 0.54[a]	3.21 ± 0.37[a,b]	3.54 ± 0.16[b]	4.10 ± 0.57[b,c]
α-acid/PAPD	2.39 ± 0.45[a]	2.43 ± 0.15[a]	2.19 ± 0.43[a]	2.22 ± 0.24[a]	2.32 ± 0.28[a]	1.90 ± 0.78[a]
β-acid	2.18 ± 0.60[a]	2.25 ± 0.99[b]	2.90 ± 0.34[a]	3.53 ± 0.20[b]	4.04 ± 0.16[b]	4.17 ± 0.08[c]
β-acid/PAPD	2.32 ± 0.58[a]	2.08 ± 0.43[a]	2.20 ± 0.46[a]	2.08 ± 0.94[a]	2.13 ± 0.23[a]	2.09 ± 0.95[a]

[a–c]Means with different letters within a column are significantly different ($P < 0.05$).
[1]Number of observations in each storage per inhibitor, n = 6.
[2]Inhibitors as in Table 1.

Table 4. Population[1] of *L. monocytogenes* at 4 and 7°C on aerobic-packaged deli-style turkey with various inhibitors[2] and sliced after 60 d of storage whole sticks.

	Population of *Listeria monocytogenes* (log CFU/g) on storage day:					
Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Storage at 4 °C						
CTR	2.76 ± 0.23[a]	2.73 ± 0.24[a]	2.84 ± 0.39[a]	2.98 ± 0.05[a]	3.06 ± 0.04[a]	3.21 ± 0.06[a]
PLSD	2.67 ± 0.45[a]	2.70 ± 0.33[a]	2.66 ± 0.26[a]	2.59 ± 0.27[a]	2.67 ± 0.28[a]	2.66 ± 0.22[a]
PAPD	2.78 ± 0.35[a]	2.69 ± 0.31[a]	2.61 ± 0.34[a]	2.62 ± 0.31[a]	2.66 ± 0.26[a]	2.53 ± 0.13[a]
α-acid	2.73 ± 0.34[a]	2.72 ± 0.26[a]	2.59 ± 0.36[a]	2.56 ± 0.28[a]	2.65 ± 0.37[a]	2.74 ± 0.24[a]
α-acid/PAPD	2.78 ± 0.11[a]	2.50 ± 0.35[a]	2.54 ± 0.34[a]	2.63 ± 0.38[a]	2.56 ± 0.28[a]	2.67 ± 0.36[a]
β-acid	2.66 ± 0.26[a]	2.59 ± 0.41[a]	2.66 ± 0.55[a]	2.83 ± 0.63[a]	3.06 ± 0.65[a]	3.27 ± 0.86[a]
β-acid/PAPD	2.78 ± 0.19[a]	2.61 ± 0.32[a]	2.47 ± 0.29[a]	2.45 ± 0.18[a]	2.59 ± 0.26[a]	2.56 ± 0.17[a]
Storage at 7 °C						
CTR	2.76 ± 0.23[a]	2.92 ± 0.17[a]	3.00 ± 0.03[a]	3.29 ± 0.13[a]	3.65 ± 0.01[b]	4.08 ± 0.16[b]
PLSD	2.67 ± 0.45[a]	2.63 ± 0.16[a]	2.68 ± 0.19[a]	2.73 ± 0.21[a]	2.76 ± 0.23[a]	2.84 ± 0.28[a]
PAPD	2.78 ± 0.35[a]	2.73 ± 0.33[a]	2.68 ± 0.24[a]	2.64 ± 0.19[a]	2.68 ± 0.24[a]	2.75 ± 0.25[a]
α-acid	2.73 ± 0.34[a]	2.76 ± 0.26[a]	2.68 ± 0.07[a]	2.79 ± 0.20[a]	2.84 ± 0.10[a]	3.05 ± 0.09[a,b]
α-acid/PAPD	2.78 ± 0.11[a]	2.72 ± 0.32[a]	2.69 ± 0.24[a]	2.71 ± 0.18[a]	2.72 ± 0.23[a]	2.80 ± 0.33[a]
β-acid	2.66 ± 0.26[a]	2.73 ± 0.38[a]	3.00 ± 0.80[a]	3.20 ± 1.04[a]	3.50 ± 1.23[b]	3.87 ± 1.32[b]
β-acid/PAPD	2.78 ± 0.19[a]	2.73 ± 0.26[a]	2.58 ± 0.09[a]	2.70 ± 0.28[a]	2.63 ± 0.08[a]	2.67 ± 0.18[a]

[a,b]Means with different letters within a column are significantly different ($P < 0.05$).
[1]Number of observations in each storage per inhibitor, n = 6.
[2]Inhibitors as in Table 1.

significantly more *Listeria* growth than control and hop β-acids ($P < 0.05$), with intermediate inhibition observed for hop α-acids (Table 4).

In liquid media, a single addition of α-acids was reported to allow *Listeria* to grow significantly higher than α-acid/PAPD combination during 6 d of storage at 7°C (Sansawat et al., 2016). In ready-to-eat meats, Seman et al. (2004) indicated that the amount of 20,000 ppm hop β-acid is required to reduce *Listeria* populations by 2.1 log CFU/package, whereas a complete inhibition of *L. monocytogenes* was observed after combining 3.0 ppm hop β-acid, 1.0% potassium lactate, and 0.25% sodium diacetate (Shen and Sofos, 2008).

In turkey slurry, Sansawat et al. (2018) reported that the concentration of α-acid > 100 ppm or β-acid > 500 ppm was minimally required to inhibit *L. monocytogenes* when the hop acids were added as a single inhibitor and stored at 7°C or less. Results from the current study showed that the combination of hop β-acids and PAPD demonstrated more stable and stronger inhibition than any single addition of hop acids or PLSD especially at an elevated storage temperature of 7°C/60 d (Table 2).

CONCLUSIONS

Antilisterial effects of hop acids have been assessed frequently in liquid media, occasionally in surface application, and rarely in product formulation. Formulation of antibacterial additives into meat batter is more desirable than surface application due to the assurance of inhibition externally and internally. Results of the

current study indicated that hop acids formulation at 5 ppm did not inhibit *Listeria* growth nor induce any synergistic effects in deli-style turkey at 4°C, regardless of storage date. However, the formulation of hop β-acid/PAPD showed more stable and effective inhibition than hop acids and PLSD alone, with an intermediate inhibition for PAPD, in vacuumed storage at 7°C/60 d. Additional research is required to induce synergistic effects at 4°C using the combination of hop acids 5 to 50 ppm and organic acids 0.1 to 0.2% sodium diacetate with no sensory objections. It has been reported that bitter taste was detected above 50 ppm hop acids and sour flavor was observed at 0.2 to 0.3% sodium diacetate (Millis and Schendel, 1994; Stekelenburg and Kant-Muermans, 2001; Islam et al., 2002).

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