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## Antimicrobial Efficacy of *Alpinia galanga* (Linn.) Swartz Flower Extract Against *Listeria monocytogenes* and *Staphylococcus aureus* in a Ready-to-Eat Turkey Ham Product

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**Abstract:** The pH, objective color and antimicrobial efficacy of *Alpinia galanga* (Linn.) Swartz Flower Extract (GFE) in a ready-to-eat turkey ham product inoculated with *Listeria monocytogenes* and *Staphylococcus aureus* were evaluated. Ham treatments included positive control (inoculated with *Listeria monocytogenes* and *Staphylococcus aureus* cocktail only), 0.5% GFE incorporated into meat batter prior to cooking, 1.0% GFE incorporated into meat batter prior to cooking, 0.5% GFE applied to surface of cooked ham after cooled and 1.0% GFE applied to surface of cooked ham after cooled. All samples were inoculated after cooking, then vacuum packaged, stored at 4±1°C for 28 days and analyzed at 1-week intervals. Galangal flower extract exhibited antimicrobial properties and resulted in reductions ( $p>0.05$ ) of up to 1.00 log cfu/g in *S. aureus* and *L. monocytogenes* when compared to the inoculated control. Galangal extract had no adverse effects on objective color and pH.

**Key words:** Galangal flower extract, *Listeria monocytogenes*, *Staphylococcus aureus*, ready to eat products, Turkey ham

### INTRODUCTION

*Listeria monocytogenes* and *Staphylococcus aureus* have been implicated in ready-to-eat (RTE) poultry and meat products. Increasing demand and consumption of RTE foods have resulted in significant economic importance for the RTE food industry (Cutler *et al.*, 2003) and unfortunately, in a higher number of outbreaks. Analysis of eleven years of foodborne outbreak data and consultations with peer-reviewed experts resulted in *Listeria* spp. being ranked third in the top 10 pathogen-food combinations in terms of annual disease burden in deli meats (Batz *et al.*, 2011). The associated costs of illness, hospitalizations and deaths were \$1.09 million, 595 and 104, respectively (Batz *et al.*, 2011; Scallan *et al.*, 2011; Mead *et al.*, 1999). Although many of the RTE products are cooked to eliminate pathogens such as *L. monocytogenes*, *S. aureus* and *Salmonella* spp., recontamination could possibly occur from cross-contamination of infected raw products, processing equipment, food handlers, post-processing, packaging or a combination of these (Farber and Peterkin, 1999; Tassou *et al.*, 2007; Lindenberger, 2011; Cochran, 2011).

*L. monocytogenes* can grow to significant numbers at refrigeration temperatures within hours or survive without reproduction at 4°C (WHO and FAO, 2004). The organism can grow well in sterile minced meat at 4°C or naturally contaminated minced meat at 20°C ((International Commission on Microbiological Specification for Foods) ICMSF, 1996), survive for

several weeks at -18°C in various food substrates (Golden *et al.*, 1988) and has high tolerance for salt and the ability to survive in foods that have low pH (Mbata, 2010; Bell and Kyriakides, 2005). In addition to RTE foods, the organism has been isolated in approximately 6% of poultry carcass rinses and in 31% of ground poultry meat (USDA-FSIS, 1997). The presence of *S. aureus* in poultry products is an indication of improper handling techniques. *S. aureus* is capable of producing exotoxins that are responsible for staphylococcal food poisoning (Dinges *et al.*, 2000).

To reduce the incidents of outbreaks, illnesses and recalls due to pathogens such as *L. monocytogenes* and *S. aureus* in RTE poultry products, researchers have investigated various approved ingredients such as nisin, chlorine, organic acids and other agents for antimicrobial properties (Sharma *et al.*, 2012; Ruiz *et al.*, 2010; Nattress *et al.*, 2001; Marsden *et al.*, 2000). In addition, researchers have investigated natural herbs and spices for antimicrobial properties against Gram Negative and Gram-positive organisms. Although spices and herbs are commonly used for flavoring, in recent years, researchers have documented that spices and herbs, such as clove, cinnamon, oregano and rosemary, have antimicrobial and antioxidant properties (Weerakkody *et al.*, 2010; Cheah and Gan, 2000; Hsu *et al.*, 2010; Rahman *et al.*, 2009).

In addition, *Alpinia galanga* (Linn.) Swartz is an herb that has been commonly used in Indian and Asian countries for medicinal uses and as a flavorful spice in foods (Hsu

*et al.*, 2010). Parts of the distinctive herb are being viewed as an effective natural antimicrobial that will retard oxidative rancidity and minimize or eliminate Gram-positive pathogens in RTE foods (Cheah and Gan, 2000; Cheah and Hasim, 2000; Hsu *et al.*, 2010). Oonmetta-Aree *et al.* (2006) evaluated *A. galanga* extracted in 100% ethanol to determine its antimicrobial properties against *S. aureus* and *E. coli* using the agar disc diffusion assay. The galangal extract had the strongest inhibitory effect against *S. aureus*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for galangal extract was 0.325 and 1.3 mg/ml, respectively. Transmission electron microscopy demonstrated that the galangal extract caused both outer and inner membrane damage and cytoplasm coagulation. The objectives of this study were to investigate the antimicrobial efficacy of *Alpinia galanga* (Linn.) Swartz flower extract (GFE) in a ready-to-eat turkey ham inoculated with *L. monocytogenes* and *S. aureus* and ascertain its effect on pH and objective color.

## MATERIALS AND METHODS

**Preparation, cultivation and storage of inoculum:** Reference strains of *L. monocytogenes* ATCC 51772 (Serotype 1/2a) and *S. aureus* ATCC 8095 were obtained from ABC Research Corporation in Gainesville, FL and used as the inoculum in this study. Each strain was received on tryptic soy agar (TSA, DF 0369-17-6, Difco Laboratories, Detroit, MI) slants and transferred to four test tubes containing 10 mL of tryptic soy broth (TSB, DF 0370-17-3, Difco Laboratories, Detroit, MI) using a sterile disposable 3 mm inoculation loop. The tubes were incubated at 35°C for 24 h. After incubation, the cultures were poured into sterile 15 mL centrifuge tubes and centrifuged (RC-5 Super speed Centrifuge, Sorvall SS-34 Rotor, Dupont Instruments, Newton, CT) at 5000 rpm for 10 min. The supernatant was discarded and the pellets were resuspended in 10 mL of deionized/distilled water and recentrifuged. The supernatant was discarded and the pellets were resuspended in 1 mL of 3% TSB with 30% glycerol in a 2 mL cryovial (Cat. No. 03-374-2, Corning Incorporated, Corning, NY), stored at -45°C and used as the stock culture for the inoculation studies. A total of four vials were prepared.

Twenty-four hours prior to conducting the study, one tube of each of the individual strains was removed from the freezer and allowed to thaw for 10 min. A loopful of the cultures from each strain was transferred and mixed into a test tube containing 10 mL of 3% TSB, vortexed and incubated at 35°C for 24 h. After incubation, the cultures were centrifuged at 5000 rpm for 10 min and washed with 0.1% sterile buffered peptone water (BPW, Cat. No. DF O1897-17-4, Difco Laboratories, Detroit, MI) and serially diluted with BPW to concentrations of  $10^{-1}$ - $10^{-8}$ , plated on duplicate TSA plates and incubated for 24 h at

32°C. The resulting colony forming units were enumerated and the aliquot of the inoculum needed to yield between 5-6 log cfu/g of the inoculum was determined.

**Preparation of galangal flower extract for turkey ham samples:** Fresh galangal flowers were obtained from a local grower in Alachua County, FL and utilized in this study. The galangal flowers were dried using a vacuum drying oven and pulverized to produce a powder (Hsu *et al.*, 2010). Ten g of the galangal flower powder were combined with 150 mL of 190-proof ethanol (moderately polar, spectrophotometric grade, Acros Organics, Fair Lawn, NJ, USA) for extraction and allowed to agitate on a shaker at room temperature for 24 h. After extraction, samples were filtered through Whatman No. 1 filters (Whatman International Ltd., Maidstone, UK) using Büchner funnels to obtain clear filtrates. All filtrates were dried under reduced pressure at 40°C using a rotary evaporator (Büchi, Labortechnik AG, Flawil, Switzerland). The dried extract was reconstituted in ethanol solvent by adding 6mL of ethanol to the flask to obtain a stock solution of 300 mg/mL. The Galangal Flower Extract (GFE) was then sterilized by filtration through a 0.45 µm disc filter (Millipore, Bilerica, MA, USA) and stored in sterile vials at -20°C until used.

**Sample preparation, inoculation and treatment:** Commercially available case ready ground turkey meat was purchased from a local supermarket as soon as the shipment arrived at the store and used in this study. The ground turkey was labeled with a sell-by date of at least 30 days by the producer. The ground turkey was transported to the research laboratory in insulated coolers, stored at 4±1°C and used within 24 h. The ground turkey was portioned into 7 aliquots of 150 g each and treated in the following manner.

**Treatments included the following:**

- Cooked ham meat mixture, no GFE+*L. monocytogenes* and *S. aureus* cocktail inoculum (positive control)
- Combine 0.5% GFE+meat mixture, cook, cool, inoculate (effect of cooking and 0.5% GFE)
- Combine 1.0% GFE+meat mixture, cook, cool, inoculate (effect of cooking and 1.0% GFE)
- Cook meat mixture, cool, inoculate, treat with 0.5% GFE (effect of adding 0.5% GFE after cooking)
- Cook meat mixture, cool, inoculate, treat with 1.0% GFE (effect of adding 1.0% GFE after cooking)

The ground turkey was blended with salt (1.5%), sucrose (0.75%), sodium tripolyphosphate (0.4%), modern cure (based on 113.36g per 45.5 kg meat), sodium erythorbate (550 ppm equivalent) and water

(10%). The blended mixtures were processed as described in the treatments above. The cooking process included placing the treatments into whirl pak bags, cooking to internal temperature of 74°C and cooling to ambient temperature. The ham samples were inoculated with 100 µL of the 10<sup>8</sup> cfu/ml inoculum and allowed to stand at room temperature for 20 min to facilitate bacterial attachment. All samples were aseptically placed into pre-labeled vacuum bags (FoodSaver bags, T150-00011-002, 164.232 cc/m<sup>2</sup>/24 h @ 23C, 0.334 cc/m<sup>2</sup>/24 h @ 23C) and stored at 4±1°C for 28 days. Duplicate samples per treatment were analyzed after 0, 7, 14, 21 and 28 days of storage for *L. monocytogenes*, *S. aureus*, pH and objective color.

**Microbiology and pH:** Duplicate 11 gram samples were transferred aseptically from the vacuum bag to a sterile stomacher bag (01-002-44, Fischer Scientific) containing 99 mL of sterile 0.1% BPW (DF O1897-17-4, BD Diagnostics, Sparks, MD) and agitated for 60 s. Appropriate serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 ml of sterile BPW. A 100 µl aliquot of the dilutions was pipetted onto pre-poured modified Oxford agar plates (DF0225-17-0, BD Diagnostics) with Oxford media supplement (DF0214-60-9, BD Diagnostics) for *L. monocytogenes* and Mannitol Salt agar plates (MSA, R453902, Remel Inc., Lenexa, KS) for *S. aureus*. All plates were incubated for 48 h at 35±1°C. After incubation, colony forming units from each plate were counted, averaged and reported as log colony forming units per gram (log cfu/g).

Immediately after microbiology analyses, pH was recorded for each sample (Accumet Basic pH meter, Model No. AB15, Fisher Scientific, Fair Lawn, NJ). The pH probe was placed into the sample homogenate and allowed to equilibrate for 1 min before the reading was taken. All pH readings were performed in duplicate.

**Objective color analysis:** Duplicate samples per treatment were evaluated for color using the Miniscan XE plus Hunter Colorimeter (Cat. No. 4-320, Fischer Scientific). The colorimeter was calibrated on each sampling day using standard black and white tiles as recommended by the manufacturer. The samples were scanned prior to opening the package to avoid contamination of the samples prior to microbiological analysis. In order to account for the packaging material, a single sheet of the vacuum package film was placed over the calibration tiles. Each package was scanned to yield L\*, a\* and b\* values. The two L\*, a\* and b\* values for each treatment were averaged using the colorimeter. The L\* value measures the lightness/whiteness of a sample on a scale from 100 (white) to 0 (black). The a\* and b\* values have no numerical limits but a positive a\* value is a measure of the redness in the sample and

negative a\* value is a measure of greenness. A positive b\* value is a measure of the yellowness in the sample and negative b value is a measure of blueness (Hunter Lab., 2001).

**Data analysis:** A complete block design with five treatments, two replications, five storage periods and three trials were employed. A total of 150 samples were analyzed for pH, microbiology and color. SAS General Linear Model, Least Squared Means (LSM) and Tukey's pairwise comparison were used to determine the differences among treatments, storage days and treatment by storage day interaction. Data were significant at α = 0.05.

**RESULTS AND DISCUSSION**

**Staphylococcus aureus:** Although not significant, *S. aureus* counts for Treatments 2 and 3 were one log lower (p>0.05) than the positive control on day 0 (Table 1). *S. aureus* counts remained similar (p>0.05) through 28 days storage for all treatments which revealed that although *S. aureus* survived during storage, it did not proliferate.

**Listeria monocytogenes:** One log reduction was revealed in *L. monocytogenes* counts for Treatments 2 and 3 on 14 and through 28 days storage when compared to the control (although not always significant) (Table 2). The treatment\*day interaction (p<0.05) was due largely to the increase (p<0.05) in *L. monocytogenes* counts during 28 days storage for hams in treatments 1, 2 and 4 when compared to Day 0. In contrast, hams in treatments 3 and 5 which were treated with 1.0% GFE had similar (p>0.05) *L. monocytogenes* counts through 28 days storage when compared to day

Table 1: Least square means for *Staphylococcus Aureus* on turkey hams inoculated with *Listeria Monocytogenes* and *Staphylococcus Aureus*, treated with *Alpinia Galanga* (Linn.) Swartz Flower Extract and stored at 4±1°C for 28 Days

<i>Staphylococcus aureus</i> (Log cfu/g)					
Storage days					
Treatment*	0	7	14	21	28
1	5.98 <sup>a,u</sup>	5.88 <sup>a,u</sup>	5.79 <sup>a,u</sup>	4.26 <sup>a,u</sup>	5.28 <sup>a,u</sup>
2	4.96 <sup>a,u</sup>	4.94 <sup>a,u</sup>	5.28 <sup>a,u</sup>	4.78 <sup>a,u</sup>	5.08 <sup>a,u</sup>
3	4.96 <sup>a,u</sup>	5.99 <sup>a,u</sup>	5.22 <sup>a,u</sup>	5.80 <sup>a,u</sup>	4.94 <sup>a,u</sup>
4	5.56 <sup>a,u</sup>	6.33 <sup>a,u</sup>	5.22 <sup>a,u</sup>	6.36 <sup>a,u</sup>	5.71 <sup>a,u</sup>
5	5.45 <sup>a,u</sup>	5.30 <sup>a,u</sup>	5.49 <sup>a,u</sup>	6.02 <sup>a,u</sup>	5.35 <sup>a,u</sup>
SEM	0.39	0.41	0.38	0.50	0.28

<sup>a</sup>Means within a column lacking a common superscript differ (p<0.05).

<sup>u</sup>Means within a row lacking a common superscript differ (p<0.05).

\*1 = cook meat, no GE+Inoculum (positive control), 2 = 0.5% GE, cook+Inoculum, 3 = 1.0% GE, cook+Inoculum, 4 = cook, inoculate, add 0.5% GE (post cook-treatment), 5 = cook, inoculate, add 1.0% GE+Inoculum (post cook treatment). SEM = Standard Error Mean

Table 2: Least square means for *Listeria monocytogenes* on Turkey Hams inoculated with *Listeria Monocytogenes* and *Staphylococcus Aureus*, treated with *Alpinia Galanga* (Linn.) Swartz Flower Extract and stored at 4±1°C for 28 Days

Treatment*	<i>Listeria monocytogenes</i> (Log cfu/g)				
	Storage days				
	0	7	14	21	28
1	5.53 <sup>a,w</sup>	5.82 <sup>a,vw</sup>	6.70 <sup>a,uv</sup>	6.98 <sup>a,u</sup>	7.23 <sup>a,u</sup>
2	5.32 <sup>a,v</sup>	5.95 <sup>a,u</sup>	5.68 <sup>a,u</sup>	5.87 <sup>a,u</sup>	6.13 <sup>ab,u</sup>
3	5.37 <sup>a,v</sup>	5.72 <sup>a,v</sup>	5.23 <sup>a,u</sup>	5.80 <sup>a,u</sup>	5.90 <sup>b,u</sup>
4	5.59 <sup>a,v</sup>	5.72 <sup>a,v</sup>	6.88 <sup>a,uv</sup>	6.62 <sup>a,uv</sup>	7.06 <sup>ab,u</sup>
5	5.51 <sup>a,uv</sup>	4.93 <sup>a,v</sup>	5.95 <sup>a,uv</sup>	5.91 <sup>a,uv</sup>	6.73 <sup>ab,u</sup>
SEM	0.50	0.45	0.38	0.28	0.10

<sup>a,b</sup>Means within a column lacking a common superscript differ (p<0.05).

<sup>u,v,w</sup>Means within a row lacking a common superscript differ (p<0.05). \*1 = cook meat, no GE+Inoculum (positive control), 2 = 0.5% GE, cook+Inoculum, 3 = 1.0% GE, cook+Inoculum, 4 = cook, inoculate, add 0.5% GE (post cook-treatment), 5 = cook, inoculate, add 1.0% GE+Inoculum (post cook treatment). SEM = Standard Error Mean

0. The ability of *L. monocytogenes* to grow in refrigerated vacuumed packaged ham was also reported by Glass and Doyle (1989) and Todar (2012).

**pH:** The pH values were similar (p>0.05) among treatments and over time from days 0-28 (Data not shown). The pH values ranged from 6.87-7.04 for all treatments. Barbut and Mittal (1993) reported a pH value of 6.50 for fresh ground turkey thigh meat. In this study, analysis of fresh ground turkey meat and cured cooked turkey ham revealed slightly higher pH values of 6.83 and 6.92, respectively. These findings revealed that the GFE had no adverse effect on the pH of the RTE turkey hams produced in this study. Glass and Doyle (1989) inoculated processed meat with 5 log cfu/gram of *L. monocytogenes* and demonstrated that the microorganism grew well on meat with pH values near or above pH 6.

**Objective color:** L\* Values. In general, the L\* values were similar (p>0.05) among all treatments during 28 days storage (Data not shown). The L\* values ranged from 40.00-50.00 for all treatments which indicated that the GFE had no adverse effect on the lightness of the RTE product. The L\* values for turkey ham (Zhu *et al.*, 2004) and cured beef sausage and pork ham (Paxton *et al.*, 2009; Zhang *et al.*, 2007) range from 50.0-64.0 which includes values obtained in this study. However, color values differ based on actual added ingredients in the product formulation and the initial pH of the meat.

**a\* Values:** The a\* values were similar (p>0.05) among all treatments during 28 days storage (Table 3). As storage time increased, the a\* values increased but not significantly (p>0.05) during storage for all treatments

Table 3: Least square means for a\* and b\* color values for turkey hams inoculated with *Listeria monocytogenes* and *Staphylococcus aureus*, treated with *Alpinia galanga* (linn.) Swartz flower extract and stored at 4±1°C for 28 days

Color attribute	Treatment <sup>1</sup>	Storage days				
		0	7	14	21	28
a*	1	8.46 <sup>a,u</sup>	9.00 <sup>a,u</sup>	10.53 <sup>a,u</sup>	9.03 <sup>a,u</sup>	10.04 <sup>a,u</sup>
	2	6.88 <sup>a,u</sup>	10.22 <sup>a,u</sup>	9.38 <sup>a,u</sup>	9.09 <sup>a,u</sup>	8.26 <sup>a,u</sup>
	3	6.90 <sup>a,u</sup>	8.10 <sup>a,u</sup>	8.74 <sup>a,u</sup>	9.30 <sup>a,u</sup>	8.72 <sup>a,u</sup>
	4	7.40 <sup>a,u</sup>	7.89 <sup>a,u</sup>	8.24 <sup>a,u</sup>	9.49 <sup>a,u</sup>	9.40 <sup>a,u</sup>
	5	7.68 <sup>a,u</sup>	7.50 <sup>a,u</sup>	7.01 <sup>a,u</sup>	7.91 <sup>a,u</sup>	8.40 <sup>a,u</sup>
	SEM	1.06	0.77	1.37	1.07	0.95
b*	1	8.93 <sup>a,v</sup>	5.06 <sup>a,v</sup>	5.72 <sup>a,v</sup>	5.34 <sup>a,v</sup>	5.60 <sup>a,v</sup>
	2	8.83 <sup>a,v</sup>	6.23 <sup>a,v</sup>	5.57 <sup>a,v</sup>	5.40 <sup>a,v</sup>	5.07 <sup>a,v</sup>
	3	9.80 <sup>a,v</sup>	6.01 <sup>a,v</sup>	6.57 <sup>a,v</sup>	6.43 <sup>a,v</sup>	6.20 <sup>a,v</sup>
	4	9.39 <sup>a,v</sup>	5.21 <sup>a,v</sup>	5.05 <sup>a,v</sup>	5.70 <sup>a,v</sup>	5.83 <sup>a,v</sup>
	5	9.62 <sup>a,v</sup>	6.08 <sup>a,v</sup>	5.38 <sup>a,v</sup>	6.01 <sup>a,v</sup>	6.84 <sup>a,uv</sup>
	SEM	1.15	0.62	0.90	0.44	0.59

<sup>a</sup> Means within a column and within the same color attribute lacking a common super differ (p<0.05).

<sup>u,v</sup> Means within a row and within the same color attribute lacking a common super differ (p<0.05).

<sup>1</sup> 1 = cook meat, no GE+Inoculum (positive control), 2 = 0.5% GE, cook+Inoculum, 3 = 1.0% GE, cook+Inoculum, 4 = cook, inoculate, add 0.5% GE (post cook-treatment), 5 = cook, inoculate, add 1.0% GE+Inoculum (post cook treatment). SEM = Standard Error Mean

when compared to day 0. The a\* values in this study ranged from 6.88-10.53. The data suggested that a\* values were not adversely affected by the GFE. The a\* values for turkey ham (Ruiz *et al.*, 2009; Zhu *et al.*, 2004) and cured beef sausage and pork ham (Paxton *et al.*, 2009; Zhang *et al.*, 2007) range from 7.25-20.9 which includes values obtained in this study. As previously discussed, color values differ based on actual added ingredients in the product formulation and the initial pH of the meat.

**b\*Values:** The b\* values were similar (p>0.05) among all treatments (Table 3). The treatment \* time interaction was attributed primarily to decreases (p<0.05) in b\* values as storage time increased for all treatments. A decrease in b\* values for cured poultry and meat products is indicative of formation of nitrosometmyoglobin which is the onset and/or progression of brown pigmentation in the product. The b\* values for turkey ham (Ruiz *et al.*, 2009) and cured beef sausage and pork ham (Paxton *et al.*, 2009; Zhang *et al.*, 2007) range from 7.05-8.02 which includes all b\* values obtained in this study for storage days 7 through 28.

In summary, galangal flower extract exhibited antimicrobial properties and resulted in reductions (p>0.05) of up to 1.00 log cfu/g in *S. aureus* and *L. monocytogenes* counts for the turkey hams when compared to the inoculated control. Galangal extract had no adverse effects on objective color and pH. Additional research is necessary to determine the maximum usage level for galangal flower extract that will result in significant reductions of *S. aureus* and *L.*

*monocytogenes* when used in ready to eat poultry products. It will also be important to compare antimicrobial and antioxidant efficacy of galangal flower and galangal rhizome extracts in the turkey ham. Cheah and Hasim (2000) used up to 10% galangal plant rhizome extract in cooked minced beef to retard lipid oxidation and microbial growth. The galangal rhizome extract functioned to retard rancidity but exerted minimal antimicrobial properties of less than one log reduction (0.71 log cfu/gram) which was less than the reductions achieved in this study using galangal flower extract. The use of galangal flower extract in poultry and related food animal products will have significant economic and safety impacts on the food industry. The extract will result in a natural product to be used alone or in conjunction with synthetic antimicrobials.

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