ANTIMICROBIAL EFFICACY OF FLOWER EXTRACT FROM ALPINIA GALANGA *(LINN.) SWARTZ.* AGAINST *LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* IN A READY-TO-EAT TURKEY HAM PRODUCT

By

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To mother and father; thank you for the enlightenment that allowed me to strive for the best. To my family and friends, thank you for your moral support. Above all, I am grateful to God for helping me throughout this journey.

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The increase in the consumption of ready-to-eat (RTE) meats over the last decade has resulted in an increase in the number of illness outbreaks associated with these foods. The objectives of this study were to determine the antimicrobial efficacy of galangal flower extract on a RTE turkey ham inoculated with *Listeria monocytogenes* and *Staphylococcus aureus* and stored at 4°C, and ascertain the effects of the galangal extract (GE) on pH and objective color of the ham. Seven treatments were evaluated over a period of four weeks. The treatments included 1 - uncooked sample, 2 - cooked sample with no galangal extract (negative control), 3 - inoculated cooked sample (positive control), 4 - inoculated cooked sample with 0.5% galangal extract, 5 - inoculated cooked sample with 1.0% galangal extract, 6 - inoculated cooked sample with 0.5% galangal extract (post cooked), 7 - inoculated cooked sample with 1.0% galangal extract (post cooked). In treatments 3 through 5, the galangal extract was incorporated into the raw mixture and cooked. In treatments 6 and 7, the galangal extract was applied to the meat after it was cooked. After treatment, all samples were vacuum packaged, stored at 4 ± 1°C for 28 days, and analyzed at 1-wk intervals. Samples were analyzed for pH, total plate count, *L. monocytogenes, S. aureus,* and color (L*A*B*).

The data revealed that the GE treatments resulted in no significant ($P > 0.05$) log reductions against *L. monocytogenes and S. aureus*. The counts for both organisms were similar ($P > 0.05$) for all samples treated with 0.5 and 1.0% GE (treatments 4 through 7) when compared to the positive control. However, turkey samples treated with 0.5% GE prior to cooking resulted in a one log cfu/gram reduction ($P > 0.05$) in *S. aureus* on day 0, and a one log cfu/g reduction ($P > 0.05$) in *L. monocytogenes* on days 0 when compared to the positive control. Although not significantly different ($P > 0.05$) *S. aureus* counts for treatments 4 and 5 were lower ($P > 0.05$) than the positive control (treatment 3) on day 0. Treatment 4 also had lower counts ($P > 0.05$) than treatment 6 of 0.40 or more log cfu/g for *S. aureus* on days 0, 7, 21, 28 and up to 1.87 log cfu/g in *L. monocytogenes* on all days. Treatment 5 had lower counts ($P > 0.05$) compared to treatment 7 of 0.20 or more log cfu/g for *S. aureus* on days 0, 14. 21, 28 and 0.20 or more log cfu/g for *L. monocytogenes* on days 0, 14, 28. The raw samples (treatment 1) and cooked untreated samples (treatment 2) were lower ($P > 0.05$) than all treated samples through the 28 days of storage. This study was also used as a model to determine the best application method for the GE. Based on the bacterial counts for total plate count, *S. aureus*, and *L. monocytogenes*, the galangal extract would be more effective as an ingredient incorporated into the meat mixture prior to cooking compared to applying the GE on the surface of the cooked meat as a post processing treatment.

CHAPTER 1
INTRODUCTION

Listeria monocytogenes and *Staphylococcus aureus* are two pathogens that are widely found in ready-to eat (RTE) meat products. These organisms possess characteristics that will allow them to survive or even multiply. *L. monocytogenes* has high tolerance for salt and the ability to grow in foods that have low pH (Bell and Kyriakides, 2005). *S. aureus* is capable of producing exotoxins that are responsible for staphylococcal food poisoning (Dinges et al., 2000). The post contamination of *S. aureus* occurs solely during processing and packaging (Tassou et al., 2007).

Increasing demand and consumption of RTE foods over the past two decades resulted in great economic importance for the RTE food industry (Cutler et al., 2003). However, the increase in the consumption of RTE foods has resulted in higher numbers of outbreaks involving these types of food products. The outbreaks have resulted from cross-contamination of contaminated raw products, processing equipment or food handlers, or a combination of the three. Although, many of the RTE products are cooked to eliminate pathogens such as *L. monocytogenes*, *S. aureus*, and *Salmonella*, recontamination could possibly occur during post-processing and packaging (Farber and Peterkin, 1999). These organisms have raised concerns in the food industry due to their mortality rates and implication in numerous cases. In 1999, it was reported by the Centers for Disease Control and Prevention (CDC) that *L. monocytogenes* had the second highest fatality rate of 20% and the highest hospitalization rate of 90% (Gallagher, 2003). Currently, RTE meat products are often being recalled due to the contamination *of L. monocytogenes* or *S. aureus* (Lindenberger, 2011; Cochran, 2011).

To reduce the incidents of outbreaks and recalls in RTE meat products, researchers are investigating the use of natural herbs and spices that possess antimicrobial properties against

Gram-positive organisms such as *L. monocytogenes* and *S. aureus*. Spices and herbs are common for their antimicrobial and antioxidant properties, as well as their flavors in foods. Some of the commonly used spices and herbs are clove, cinnamon, oregano, and rosemary, which are considered to have strong antimicrobial activity (Weerakkody et al., 2010). Other spices and herbs are being investigated for their antimicrobial characteristics, one of which is *Alpinia galanga* (Linn.) Swartz. *Alpinia galanga* flower is an herb that has been commonly used in Indian and Asian countries as medicine and as a flavorful spice in foods (Hsu et al., 2010). Parts of the distinctive herb are being viewed as an effective natural herb that will minimize Gram-positive pathogens in RTE foods (Cheah and Gan, 2000; Hsu et al., 2010). The objectives of this project were to observe the antimicrobial efficacy of the galangal flower extract on a RTE turkey ham product inoculated with *L. monocytogenes* and *S. aureus*, and stored at 4°C, and ascertain the effects of the galangal extract on pH and objective color of the ham.

CHAPTER 2
LITERATURE REVIEW

Microbiology of Poultry

Processing Plant Conditions

Over the past few decades the consumption of poultry products has doubled, which has led to a massive increase in animal production (USDA, 2011;Seo and Bohach, 2007). Increased poultry production has raised concerns of both contaminations with human and animal pathogens by consumers and government officials. One of the most significant solutions used to solve this contamination problem was the rapid transition from handcraft operations (i.e., the use of manual tools) between the 1950s and 1960s to mechanical processing (Bolder, 1998). This change has influenced the outcome of the finished product. The guidelines of Hazard Analysis Critical Control Point (HACCP) were also another preventative measure used to control the contamination of microbiological and chemical hazards on the final product (Bolder, 1998).

As a manufacturer, one of the factors that must be considered is the design and production of the machines being used in the production of poultry products. Proper facility design will allow the production flow to avoid finished product contact with the raw materials. Placing cleaning stations throughout the plant also allows the employees and the production workers to maintain proper hygiene. This will also reduce or limit the transfer of pathogens such as *S. aureus* to the food products (Schmidt and Erickson, 2005). The same factors would apply to smaller poultry producers, to ensure that their line workers are healthy. The use of convenient machines, such as moveable equipment for easy cleaning, also plays a major role in the production aspect. Producers must take into consideration that the machines should have the capability to be cleaned efficiently, even if it requires disassembling (Goddard, 2011). Both large and small producers share the desire to ensure safety for its consumers. Food producers would

consider food safety to be a significant factor due to the possible undesirable outcomes of a recall or outbreak, which would result in tremendous economic loss and bad media exposure for the company.

Pathogenic and Spoilage Microflora

Poultry is known to have a very complex microflora, which can be found in the intestinal system based on the production methods being used. Live conventional poultry are raised in large flocks on litter floors, which can lead to contamination with pathogens such as *Salmonella, Campylobacter, Listeria, E. coli, Clostridium, and S. aureus* (Kotula and Pandya, 1995).

Spoilage Organisms in Poultry

Spoilage microorganisms such as Pseudomonads, lactic acid bacteria, and yeasts tend to be present in live animals and are potentially being transferred to their meat products. Additionally, spoilage organisms from the environment such as those in the water supply of the processing plant can also contribute to the added contamination of spoilage microorganisms. These spoilage organisms have resistant characteristics that can survive normal chlorinated water treatments. Although during some processing steps such as scalding, these organisms can be destroyed, recontamination can occur during subsequent stages throughout the processing steps because these organisms favor wet surfaces (Mead, 1989).

Problems Associated with Pathogens in Poultry

There are a number of factors that influence the contamination of poultry products. Some of these factors begin with hatching of the live bird to the final dressing of the carcass, or even further processing that occurs at the processor's establishment (Simonsen, 1989). One of the steps that are critical in these production processes is the evisceration of the bird. A challenge that many producers face is trying to avoid tearing the intestines, which would cause fecal contamination of the carcass. Another challenge would be to avoid cutting the esophagus to

prevent microbial contamination from leakage (Fanatico, 2003). These factors can lead to recalls or foodborne outbreaks in the poultry processing industry. To control pathogens such as *L. monocytogenes* and *S. aureus* in the poultry industry, techniques such as good manufacturing practices, environmental sanitation, HACCP, pasteurization, and post processing treatments are being used. Current research focuses on taking a more natural approach with the use of natural herbs and spices that will help decrease or control the growth of these organisms. These spices and herbs have antimicrobial properties that are used in the product formulation and post-cooking marinades.

Gram Negative Pathogens in Poultry

Salmonella and *Campylobacter* are the two major pathogen species that remain of greatest concern in the poultry industry. Another concern in the poultry industry is the antimicrobial resistance of these poultry related pathogens

Salmonella spp.

Most of the *Salmonella* found in poultry products has the capability of causing human infection, which can lead to acute diseases. An acute disease is a rapid onset infection. Two of the common types of *Salmonella* that are familiar in poultry products are *Salmonella* Enteritidis and *Salmonella* Typhimurium. *Salmonella* has been found in different parts of the poultry carcasses by penetrating through the bird's internal organs or deep tissue. *Salmonella* Enteritidis contamination usually takes place in the egg or during post-production cross-contamination (Simonsen, 1989).

Salmonella spp. are facultative anaerobic, Gram-negative, rod-shaped bacteria that belong to the *Enterobacteriaceae* family. The species contains approximately 2,500 serotypes but only about 10 are responsible for human illness called salmonellosis. Salmonellosis is a disease that is transmitted from animals to man, but is primarily acquired through the

consumption of contaminated food products. Classic symptoms of the disease include stomach pain, diarrhea, headache, and fever accompanied by chills. The bacteria can grow at 37°C, but some strains can grow at an elevated temperature ≤ 54°C (D'Aoust and Maurer, 2007). *Salmonella* can grow within a pH range of 3.6 to 9.5 with an optimum range of 6.5 to 7.5 (Marriott and Gravani, 2006).

Campylobacter spp.

Campylobacter is also a Gram-negative, spirally curved, motile, rod-shaped bacterium. It is a microaerophilic bacterium, which means it requires little to no oxygen to survive. The bacteria can grow in 3 to 5% oxygen and 2 to 10% carbon dioxide. *Campylobacter* can survive up to 4 weeks in 4°C under moist, reduced-oxygen conditions (Nachamkin, 2007). The two common *Campylobacter spp.* that is commonly found in the poultry industry are *Campylobacter jejuni and Campylobacter coli. Campylobacter* has been isolated primarily on the surface of the carcasses, up to 10^6 cfu/carcass. Unlike *Salmonella* that can survive in various foods such as seafood, where as *Campylobacter* prefers the gastrointestinal tract of warm-blooded animals. *Campylobacter* also tends to multiply more in microbial counts than most other pathogens (Mbata, 2010).

Gram Positive Pathogens in Poultry

Listeria monocytogenes

L. monocytogenes is a facultative anaerobic Gram-positive, non-spore forming, rod-shaped bacterium that can be found in soil, water, and vegetation (Rocourt and Buchrieser, 2007; ICMSF, 1996). Compared to Gram-negative organisms, Gram-positive organisms such as *L. monocytogenes* have a thicker peptidoglycan layer in their cell wall, which allows them to survive in an intracellular environment. The bacterium was first isolated in the 1920s from rabbits and guinea pigs; however, it was not until the early 1980s that the significance of foods

was being observed as the primary route of transmission for human exposure to *L. monocytogenes* (ICMSF, 1996; WHO and FAO, 2004).

Unlike other major foodborne microorganisms, *L. monocytogenes* can grow to significant numbers at refrigeration temperatures within hours (WHO and FAO, 2004). The organism can also survive for several weeks at -18°C in various food substrates (Golden et al., 1988) but does not survive well under acidic environments (El-Kest and Yousef, 2006). *L. monocytogenes* can sometimes survive without reproduction at 4°C, however, it can grow well in sterile minced meat at 4°C or naturally contaminated minced meat at 20°C (ICMSF, 1996). Recent observations have shown that *L. monocytogenes* survives in foods that are stored at refrigeration for extended periods of time, having potential for contamination, and providing nutrients for the organism to survive (Walls, 2005). The organism requires minimal oxygen in order to grow; therefore, it survives for long periods of time in the environment, foods, processing plants, and household refrigerators. Contamination of the organism in RTE foods normally occurs from contact with contaminated equipment such as brine chill chambers, slicers, peelers, conveyors, and packaging machines (Tompkin, 2002). *L. monocytogenes* is considered to be an environmental contaminant that has been isolated from many natural surroundings such as water, soil, sewage, mud, and animals (Donnelly et al., 1992).

***Listeria monocytogenes:* Listeriosis**

L. monocytogenes is known to cause listeriosis especially in immuno-compromised individuals, pregnant women, the elderly and infants, which are at a higher risk. This disease can result in abortions, stillbirths, septicemia, meningitis, encephalitis, and death (Nelson et al., 2004). In the US, *L. monocytogenes* is responsible for approximately 2,500 cases of listeriosis each year, 91% of which are hospitalization and 20% fatality (Mead et al., 1999; Scallan et al., 2011). Approximately one-third of those cases involve pregnant women and their unborn or

newly born infants (ICMSF, 1996). Symptoms that are common in perinatal cases include mild fever in the mother with or without gastroenteritis symptoms, but can result in major consequences such as meningitis or death for the fetus or newborns. Other symptoms that can occur in cases that are not perinatal are bacteremia, which is the presence of bacteria in the blood, and meningitis (ICMSF, 1996).

Listeria monocytogenes: Foods implicated

L. monocytogenes gained its name from its ability to infect the monocytes (white cells) in the blood (ICMSF, 1996). It is the main human pathogen of the *Listeria* genus that is responsible for a number of foodborne outbreaks of listeriosis that are related to (RTE) foods such as milk, soft-ripened cheeses, coleslaw, and vacuum-packaged meats (Ooi and Lorber, 2005).

Listeria monocytogenes is a pathogen that is prevalent in the poultry industry. The organism is the leading cause of mortality and morbidity in humans. The pathogen can be found in a variety of foods especially in poultry products. A high dose (10^9 cfu) of this organism can cause human listeriosis, which is a rare disease (Mbata, 2010). The contamination of this pathogen normally occurs through the cross-contamination of turkey or chicken in which the organism can spread to cooked and RTE foods. *L. monocytogenes* can be commonly found in natural environments and in the intestinal tract of animals. In the US, the organism has been isolated in approximately 6% of poultry carcass rinses and in 31% of poultry ground meat (USDA-FSIS, 1997). Cooking can destroy the organism; however, recontamination can occur during post-cooking techniques.

Listeria monocytogenes: Problems associated with *L. monocytogenes*

Recalls. *L. monocytogenes* usually triggers class 1 type recalls, which are defined by the Food and Drug Administration (FDA) as a situation that has an exposure to a product that can cause adverse health consequences or death (FDA, 2012). RTE meat products are being recalled

due to possible contamination of *L. monocytogenes*. In 2011, there was a total of 11 recalls issued by the USDA-FSIS due to possible *L. monocytogenes* contamination, and six of which were RTE meat products. When the organism first emerged as a public health problem associated with deli meats and other processed foods, USDA-FSIS established a "zero tolerance", no detectable level, for *L. monocytogenes* in RTE foods. Any products that contain the organism at any level would be considered adulterated under the Federal Meat Inspection Act (FMIA) or the Poultry Products Inspection Act (PPIA) (USDA-FSIS, 1998). One of the most recent meat recalls occurred in August 2011 for diced bacon products that was contaminated with the pathogen (Lindenberger, 2011). *L. monocytogenes* has also been associated with large outbreaks as well. Most of the large foodborne outbreaks of listeriosis have been associated with three serogroups of *L. monocytogenes*; 1/2a, 1/2b, or 4b (Bell, 2000; Nelson et al., 2004).

Outbreaks. Over the last decade, there have been a number of outbreaks associated with *L. monocytogenes*, which has resulted in chronic illnesses or even death in humans and more than 40 animal species (Nightingale et al., 2005). One of the earliest outbreaks associated with *L. monocytogenes* was in Germany in 1949, and was linked to the consumption of contaminated raw milk.

A multi-state outbreak that resulted in plant contamination with *L. monocytogenes* serotype 4b infected RTE hot dogs and deli meat that was produced by Sara Lee. This outbreak resulted in a total of 21 deaths, 15 of which were adults and the remaining 6 were miscarriages/stillbirths. The company issued a voluntary recall on 35,000,000 lbs of meat, but was only able to recover 5,918,795 lbs (USDA-FSIS, 1998; CDC, 1999).

L. monocytogenes serotype 4b has been the most isolated serotype in many food outbreaks. This strain was involved in a multistate outbreak involving contaminated hot dogs and

deli meat in 1998 to 1999, which led to at least 50 illnesses where six adults died and two pregnant women had spontaneous abortions (CDC, 2001). Another case that involved *L. monocytogenes* serotype 4b was a multistate outbreak in November of 2002 involving RTE turkey deli meat. This outbreak resulted in 54 illnesses. Out of the 54 reported cases, eight people died and three pregnant women had spontaneous abortions (Gottlieb, 2006). Based on the number of outbreaks that have occurred, it can be concluded that *L. monocytogenes* poses a serious threat to the RTE food supply that is produced throughout the world.

Staphylococcus aureus: Characteristics and growth conditions

The presence of *S. aureus* in poultry products is an indication of improper handling techniques. This organism is normally found on the outer surfaces of the bird as well as the sinuses and lungs. Harry (1967) found that 49% of 276 chickens and turkeys from 162 farms were positive for *S. aureus* in the skin and nasal sinuses. This poses a threat to processed products such as deboned poultry meat that is used to produce chicken franks and hot dogs that are combined with other muscle meats. With the level of contamination that is found in live birds, this organism can be found in various parts of the processed carcass. Roberts (1972) isolated *S. aureus* from 65 of 172 frozen chicken carcasses that were processed from four different farms. Some of the *S. aureus* strains that are isolated from chickens or turkeys produce enterotoxins, which are probably of human origin. The enterotoxins are initiated through the handling of cooked foods in the processing kitchen by an infected individual and subsequent temperature abuse. These toxic organisms have the ability to grow more rapidly in cooked poultry products than in raw poultry products (Mead, 1989).

Staphylococcus aureus is also a common microorganism that is associated with numerous foodborne outbreaks. *S. aureus* is a normal harmless resident flora of the human skin and nasal membranes (John, 2004). It is carried by a third of the human population. However, the growth

of *S. aureus* in foods poses a threat since many of the strains produce enterotoxins that can cause food poisoning when ingested. Sir Alexander Ogston first identified the bacterium during a microscopic examination of a pus sample removed from a patient's leg in his private laboratory (Bell, 2000). *S. aureus* is a Gram-positive, spherical bacterium. It can be transmitted into the food through many sources, including food handlers. The bacteria is unable to grow at refrigerated temperature, however, if abused it may multiply in foods such as irradiated cooked hams (Cabeza et al., 2010). *S. aureus* is a non-spore forming bacteria that is resistant to radiation (Erdman et al., 1961). *S. aureus* is also a facultative anaerobic organism that is the most common cause of staph intoxications. The organism can grow in a temperature range between 7 and 48°C and produce enterotoxin from 10 to 48°C, with an optimum enterotoxin production at 40 to 45°C. The pH for growth of the organism ranges between 4.0 and 9.8, with an optimum pH of 6.0 to 7.0 (Marriott and Gravani, 2006). *S. aureus* tends to grow in a non-competitive environment that contains high concentrations of salt (Seo and Bohach, 2007).

***Staphylococcus aureus*: Staphylococcal food poisoning**

Staphylococcal food poisoning (SFP), which is caused by *S. aureus,* is one of the most predominant causes of gastroenteritis in the world. This food poisoning results in the production of staphylococcal enterotoxins (SEs). SFP is an intoxication that does not involve infection by, and growth of, the bacteria in the host (Seo and Bohach, 2007). SFP symptoms usually take place within a few hours of ingestion of toxin-contaminated food, depending on the toxic dose. These symptoms include nausea, abdominal cramps, diarrhea, and vomiting. The symptoms of SFP do not result in fatality, however, it has been reported that there is an average of two deaths per year due to SFP (Mead et al., 1999). Some strains of the bacterium can produce exotoxin TSST-1, which is the causative agent of toxic syndrome. Other strains of *S. aureus* can produce enterotoxin, which can cause gastroenteritis in humans. Staphylococcal enterotoxins are resistant

to heat and cannot be distinguished in infected foods (Seo and Bohach, 2007). *S. aureus* organisms can be destroyed through the use of heat, 66°C for 12 min, however, the enterotoxins require heating for 30 min at 131°C to inactivate (Marriott and Gravani, 2006).

Staphylococcus aureus: Outbreaks

Outbreaks that have been associated with this organism usually result in cross-contamination of foods that are contaminated or food handlers that are infected with *S. aureus*. One of the major multistate outbreaks occurred in 1998. This outbreak resulted in 225 ill persons who consumed a contaminated ham salad (CDC, 2010).

Outbreaks that have been associated with *S. aureus* are not known to be as deadly or fatal as *L. monocytogenes.* CDC (2010) has reported a number of outbreaks that has taken place over the past decade but only a few have resulted in deaths. One of those outbreaks occurred in a nursing facility in South Dakota in July 2000. The source of the outbreak was consumption of chicken salad, which resulted in 95 ill persons. Out of the total ill persons, there were 13 people hospitalized, two of which resulted in death.

Ingredients Currently Used to Control Growth of Pathogens in Ready to Eat Meat and Poultry Products

Poultry producers are currently using a number of antimicrobial methods to reduce, retard, or eliminate the growth of *L. monocytogenes* and/or *S. aureus*. Antimicrobials such as nisin have been used to remove or reduce bacterial populations in lean and fat pork (Nattress et al., 2001) and RTE turkey-hams (Ruiz et al. 2010) through direct addition. Nisin has also been used to inhibit spoilage and pathogenic bacteria on RTE ham and bologna (Gill and Holley, 2000). Another form of antimicrobial that is commonly used in the poultry industry is chlorinated rinses. A chlorinated rinse usually contains 18-30 ppm of chlorine and must be maintained at 160ºF for approximately 2 hr (USDA-FSIS, 2006). Marsden et al. (2000) used a

combination of 1,200 ppm sodium chlorite and 0.9% citric acid on Little Smokies sausages to reduce *L. monocytogenes.* This resulted in a 1.2 log reduction of *L. monocytogenes.* The proper use of food handling and avoiding cross-contamination are good methods of prevention for *S. aureus*. To destroy the organism, proper cooking techniques must be used (Marriott and Gravani, 2006).

Natural Herbs as Antimicrobials

The use of galangal extract in food products

Alpinia galanga (L.) is a member of the flowering plant family Zingiberaceae, which is comprised of approximately 1,200 species including ginger. The plant originated in tropical parts of Asia such as India, Malaysia, and Indonesia (Raina et al., 2002; Wong et al., 2009), but has been cultivated worldwide in places such as the United States (Hsu et al., 2010). *Alpinia galanga,* commonly known as the greater galangal, is a species that is widely used in the foreign countries mentioned above in a variety of traditional cures and in foods as an ingredient. The rhizome of the plant is used as spice and food flavoring agent, whereas the leaves are consumed as vegetables (Wong et al., 2009). The rhizome is also used for medicinal purposes to treat diseases such as fungal skin infections, intestinal infections, stomachaches, diarrhea, vomiting, and rheumatism (Wong et al., 2009; Hsu et al., 2010).

The galangal plant is approximately 4-7 ft tall. It proliferates from underground rhizomes. The plant produces flowers in clusters of 2-5 and contains stems and leaves that are precisely hairy on the bottom (Staples and Kristiansen, 1999). In terms of cultivation, the plant requires sunny or moderately shady locations to grow, which limits its availability to the summer months of the year. The plant also requires fertile and moist soil for best results. Once the plant has reached maximum size, it is harvested three months after planting for market purposes (Peter, 2004).

Antimicrobial properties of galangal extract

The rhizomes of *A. galanga* consists of terpenoids with 1,8-cineol, which is an antimicrobial compound found in other natural herbs such as rosemary and lemon bark (Weidner et al. 2003). Terpenoid compounds are created from acetate units that are originated from fatty-acids (Cowan, 1999; Weidner et al., 2003). A number of studies have shown that the greater galangal plant has antimicrobial properties. Some of which were performed using the agar disc diffusion method for potential antimicrobial activity against *L. monocytogenes* and *S. aureus* (Hsu et al., 2010). Hsu et al. (2010) used the agar disc diffusion method to determine antimicrobial activities of oven-dried or freeze-dried galangal flowers extracted in 190-proof ethanol or hexane against *Salmonella*, *E. coli* O157:H7, *L. monocytogenes, S. aureus*, and *Shigella.* Pure bacterial cultures were swab-inoculated on to surfaces of pre-hardened Mueller-Hinton agar. Discs infused with the galangal extract solution were then placed on the surface of the incubated plates and incubated at 37°C for 18-24 hr. Results showed that the galangal extracted in the ethanol showed a broad spectrum of antimicrobial activity against Gram-positive bacteria, but showed little to no antimicrobial activity against the Gram-negative bacteria. Amongst all of the bacteria that were being tested in this study, *S. aureus* and *L. monocytogenes* showed the most sensitivity against the test extracts. The oven-dried flowers extracted in ethanol showed an inhibition zone that ranged from 26 to 31 mm. However, a larger inhibition zone was illustrated in *L. monocytogenes* with the freeze-dried samples extracted with hexane. This study also showed that there was a higher yield percentage of the extracts from the oven-dried samples than the freeze-dried samples. The oven-dried samples produced in ethanol had a higher yield than those extracted using hexane.

The rhizome of *A. galanga* has also been shown to be effective in certain meat products. In a study conducted by Cheah and Gan (2000) to observe the antioxidant properties of *A.*

galanga in minced beef, it was observed that there were antimicrobial activities in cooked beef instead of the raw beef. The study mentions that there was a 0.71 log cfu/g reduction in the total aerobic bacteria plate count.

The type of solvent used to extract the plant plays a major role in its antimicrobial activity. Extraction of the galangal plant using hexane showed greater results than the extraction using ethanol or water, which could be due to its non polar properties (Hsu et al., 2010; Weerakkody et al., 2010). Other studies have also shown that *S. aureus*, a Gram-positive organism, is more sensitive to the galangal extract than Gram-negative organisms such as *E. coli*. The results obtained by Weerakkody et al. (2011) are in agreement with other studies where the use of galangal extracts resulted in inhibition against *S. aureus* (Oonmetta-aree et al., 2006). Oonmetta-aree et al. (2006) used *A. galanga* extracted in 100% ethanol to observe its antimicrobial properties against *S. aureus* and *E. coli*. With a larger inhibition zone measured for *S. aureus* using agar disc diffusion assay, *S.aureus* was shown to be more sensitive to the extract than *E. coli*. The minimum inhibitory concentration (MIC) of galangal extract that was used to determine the sensitivity of the bacteria was 0.325 mg/ml.

The use of natural herbs in food products has been known for decades. These antimicrobials are used in foods as food preservatives to control spoilage and to prevent and control the growth of microorganisms. Natural herbs have been well known for their antimicrobial and antioxidant properties. They also have the ability to add desirable intense flavors to foods (Uhl, 2000). Galangal extract has been used in a wide variety of food products. It is commonly used in Thai or Asian dishes. Meat and poultry producers are making the attempt to use the natural herbs in their products due to its effective antimicrobial properties against food borne pathogens such as *L. monocytogenes* and *S. aureus*. Cheah and Gan (2000) conducted a

study that revealed the effects of galangal extracted in acetone on raw minced beef samples evaluated for storage stability over seven days. Three different levels of galangal treatment were employed: 0.02%, 0.05%, and 0.10% (wt/wt, fat basis). The total plate counts for the raw minced beef with the galangal extract were gradually lower than the control, which contained no galangal, over the seven-day period of this study. There was a log reduction of 0.1 cfu/g over a seven-day period. This illustrates that the addition of galangal extend the shelf life of meat products. Cheah and Hasim (2000) stated that the galangal extract exhibited antimicrobial properties, which resulted in a 0.71 log reduction.

Other Natural Herbs as Antimicrobials in Food Products

Due to the higher demands for foods that are produced with minimal chemical preservatives, natural herbs have become more appealing to food producers and their consumers. This is a challenge that food producers have taken into consideration to use natural occurring food antimicrobials and antioxidants to reduce the use of chemical preservatives. Some of the most commonly used spices and herbs are rosemary, cinnamon, oregano, and cloves (Weerakkody et al., 2010). Like galangal, rosemary is a well-known herb that has antimicrobial activities against foodborne pathogens due to its compounds. Studies have shown that combinations of galangal and rosemary had synergistic antimicrobial activity against *S. aureus* and *L. monocytogenes* (Weerakkody et al., 2011) and antioxidant effects in food products.

Weerakkody et al. (2010) conducted a study to compare the antimicrobial activities of four different spices and herbs including goraka (*Garcinia quaesita*), galangal (*Alpinia galanga*), lemon iron bark (*Eucalyptus staiger-ana*) and mountain pepper (*Tasmannia lanceolata*) to three common spices including pepper (*Piper nigrum*), rosemary (*Rosmarinus officinalis*), and oregano (*Oreganum vulgare*). These spices were extracted in water, hexane, and ethanol and tested against four different foodborne pathogens: *E. coli*, *Salmonella* Typhimurium, *L.*

monocytogenes, and *S. aureus* using the agar disc diffusion and broth dilution method. Each treatment was inoculated with 10µL of the herb and spice extraction and incubated at 37°C for 18 hr.

The different solvent types showed great antimicrobial activity with the exception of *P. nigrum*, which showed little to no activity. Each herb was applied to each bacterium individually at different usage levels, and then as four different combinations. The *A. galanga* extracted in hexane showed a larger inhibition zone (34.1 mm) against *S. aureus* compared to the other bacteria that were tested. *L. monocytogenes* was inhibited by *A. galanga* hexane extract at a MIC of < 0.625 mg/ml after 24 hr, but the MIC increased to 1.25 mg/ml 48 hr later. The MIC levels of *A. galanga* against *S. aureus* were < 0.625 mg/ml and showed no difference after 24 hr. Ethanol extracts of all the spices and herbs showed antimicrobial activity against the Gram-positive bacteria, except for *P. nigrum* and *T. lanceolata.*

L. monocytogenes was inhibited by *A. galangal* extracted in hexane and *E. staigerana* extracted in ethanol. The spices and herbs had a stronger effect on the Gram-positive microorganisms than the Gram-negatives, which used higher levels of the spices and herbs. As previously discussed, this is possibly due to the differences in the cell wall between Gram-negative and Gram-positive bacteria. Unlike the Gram-negative bacteria, antimicrobial substances can penetrate through Gram-positive bacterial cell wall and attack the cytoplasmic membrane, which causes leakage of the cytoplasm. One of the most effective herbs was the *A. galanga* in the hexane and ethanol extracts against *S. aureus* and *L. monocytogenes.* This study showed that the extraction of these spices and herbs contained components that have different modes of antimicrobial actions. The study also showed that the phenolic compound had little to no effect on the antimicrobial activity of these spices and herbs. The spices and herbs that were

extracted in the ethanol and water seemed to have a higher total phenolic content than those extracted in hexane.

Further Research Based on Literature Review

This literature review revealed that natural herbs and spices have potential as antimicrobials for use in the food industry, especially in meats. The studies in this review have revealed that the use of natural spices and herbs have the ability to reduce microbial activities in certain RTE foods. Further research is needed to evaluate the antimicrobial properties of *A. galangal* in RTE meats such as turkey hams. Turkey ham is a popular meat product that is lower in fat, compared to the traditional pork ham (USDA-FSIS, 2001). This product is fabricated from boneless, turkey thigh meat with all skin and surface fat removed. The objectives of this project were to determine the antimicrobial efficacy of galangal extract on a RTE turkey ham inoculated with *L. monocytogenes* and *S. aureus,* stored at 4°C, and to ascertain the effects of the galangal extract on pH and objective color of the hams.

CHAPTER 3
MATERIALS AND METHODS

Preparation, Cultivation, and Storage of Inoculum

Two 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 hr. 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} to 10^{-8} and plated on TSA.

Preparation of Galangal Extract for Turkey Ham Samples

Dried galangal flower powdered samples, which were obtained from the galangal flowers from a local producer in Gainesville, FL, were extracted using 190-proof ethanol (moderately polar, spectrophotometric grade, Acros Organics, Fair Lawn, NJ, USA). Ten grams of the dried sample were extracted using 150 mL of ethanol and shaken on an orbital 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 extraction yield (%) was calculated using the ratio of the final yield of dried extract (g)/10 g of original dried galangal samples and converted to a percentage (%).

After the extract was completely dried, ethanol extract was reconstituted in ethanol solvent by adding 6mL of ethanol to the flask to obtain a stock solution of 300 mg/mL, yielding 6 mL of the extract. Galangal Extract (GE) was then sterilized by filtration through a 0.45 μm disc filter (Millipore, Bilerica, MA, USA) and stored in sterile vial at – 20°C. A total of two vials were prepared for this experiment (Hsu et al. 2010).

Sample Preparation, Inoculation and Treatment

Commercially available case ready ground turkey was purchased from a local supermarket as soon as the shipment arrived at the store and was used in this study. The ground turkey was labeled with a sell-by date of at least 30 day. The ground turkey packs were immediately transported to the research laboratory in transportable coolers and stored in a walk-in cooler (4 ± 1°C) and used within 24 hr. The ground turkey was divided into 7 different presterilized trays, 150 g each, to produce seven turkey ham treatments. The turkey ham treatments included the following:

1. Raw meat, no GE, no inoculum (negative control, raw meat only)
2. No GE + cook, no inoculum (effect of cooking only)
3. Cooked meat, no GE + inoculum (positive control)
4. 0.5% GE + cook, then inoculate (effect of cooking and 0.5% GE)
5. 1.0% GE + cook, then inoculate (effect of cooking and 1.0% GE)
6. Cook, then 0.5% GE, then inoculate (effect of adding 0.5% GE after cooking)
7. Cook, then 1.0% GE, then inoculate (effect of adding 1.0% GE after cooking)

The *L. monocytogenes* and *S. aureus* cocktail was used as the inoculum for Treatments 3 through 7.

Treatments 1 – 7 were formulated as outlined in Table 2-1 and mixed in pre-sterilized trays. Each tray was divided into two groups, labeled "A" or "B" and placed into sterile Ziploc bags. With the exception of Treatment 1, all samples were cooked in a water bath for approximately 30 min or until the internal temperature of the meat reached 74°C (USDA recommended). Treatment 1 was not cooked and used as a control. Once cooked, the 6 treatments were allowed to cool for 8 – 10 min at room temperature. Treatments 3 – 7 were inoculated with 0.1 μL of the 10^8 cfu/mL inoculum. Inoculated samples were left for 20 min to allow bacterial attachment to ensure final concentration of 10^4 cfu/g. Predetermined quantities of the cooked inoculated turkey ham were aseptically weighed and placed into prelabeled vacuum bags (FoodSaver bags, T150-00011-002, 164.232 cc/m2/24 hr @ 23°C, 0.334 cc/m2/24 hr @ 23°C). All samples for each treatment were then placed into individual Gallon sized Ziploc freezer bags and stored at 4 ± 1°C in a refrigerator for 28 day. Duplicate samples per treatment were analyzed after 0, 7, 14, 21, and 28 day for aerobic plate count (APC), *L monocytogenes, S. aureus*, pH, and color using a Hunter colorimeter. Aerobic plate counts were performed on day 0 to monitor sanitation and to ensure no cross contamination during sample preparation.

Microbiology, pH, and L*a*b* Color Analyses

Eleven grams of turkey ham was 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 was agitated for 60 s. The appropriate serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 mL of sterile BPW. One microliter of the dilutions was pipetted onto prepoured modified Oxford agar plates (DF0225-17-0, BD Diagnostics) with Oxford media supplement (DF0214-60-9, BD Diagnostics) for *L. monocytogenes*, mannitol salt agar plates (MSA, R453902, Remel Inc., Lenexa, KS) for *S. aureus*, and tryptic soy agar for total plate count. All plates were incubated for 48 h at 35 ± 1°C. After incubation, colony-forming units from each plate were counted, recorded, averaged, and reported as log colony-forming units per gram (cfu/g).

Prior to microbiology analyses, pH was recorded for each sample using a pH meter (Accumet Basic, Model No. AB15). 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

Vacuum-packed samples were evaluated for color using the Miniscan XE plus Hunter Colorimeter (Cat. No. 4-320, Fischer Scientific) for day 0 – 28. Each treatment contained two vacuum-packed samples that were 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 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 a negative a* value is a measure of greenness. A positive b* value is a measure of the yellowness in the sample and a negative b value is a measure of blueness (Hunter Lab, 2001).

Data Analysis

The data analysis of this experiment was designed using JMP Pro 9.0.2 (64 – bit Edition, 70108654, 2010). A complete block design with seven treatments and two replications was used

to evaluate pH, microbiological analysis, and color analysis. A total of 140 samples were analyzed for pH, microbiology, and color over a course of five weeks (0, 7, 14, 21, 28 d). To obtain the standard errors of the mean (SEM), the General Linear Model and least squared means (LSM) were used. The SEM was used to analyze the differences between the treatment means. Data was significant at $\alpha = 0.05$. The Multivariate Analysis of Variance (MANOVA) was used to determine the differences among treatments, storage days, and treatment by storage day interaction. The Tukey's pairwise comparison was used to compare the treatment means.

Table 3-1. Formulation for Turkey Hams

	No GE (g)[1]	0.5% GE (g)[2]	1.0% GE (g)[3]	Raw[4]
Meat	150	150	150	150
Salt	2.25	2.25	2.25	--
Sugar	1.13	1.13	1.13	--
Sodium Trypolyphosphate (STPP)	0.60	0.60	0.60	--
Modern Cure	0.4	0.4	0.4	--
Na Erythorbate	0.1	0.1	0.1	--
Water	15	15	15	--
Galangal Extract	--	0.75*	1.50*	--

[1]Used for Treatments 2 and 3.[2]Used for Treatments 4 (GE added prior to cooking process) and 6 (GE was added after cooking process)*.[3]Used for Treatments 5 (GE added prior to cooking process) and 7 (GE was added after cooking process)*.[4]Used for Treatment 1, which remained uncooked

CHAPTER 4
RESULTS AND DISCUSSION

The main objective of this study was to evaluate the antimicrobial efficacy of the galangal extract on a RTE turkey ham product inoculated with *L. monocytogenes* and *S. aureus*, stored at 4°C, while observing the color and pH stability of the product.

Microbiology

Total Plate Count

The data revealed significant differences ($P < 0.05$) between trials 2 and 3, and no difference between trials 1 and 2, and 1 and 3 ($P > 0.05$). Significant treatment by time, and time and treatment differences were revealed. The total plate counts contained less than 8 log cfu/g during the storage period of 4 weeks (Table 4-1, 4-2, 4-3) for all trials. In some cases, the ham samples that were treated with the galangal extract illustrated bacteriostatic properties. Bacteriostatic properties are found in agents that are used to prevent the growth of bacteria, keeping them in a stationary growth phase (Pankey and Sabath, 2004).

The difference in trials 2 and 3 indicated that there was a time effect for each treatment, a treatment effect among the different treatments, and a time by treatment interaction ($P < 0.05$). Except for day 7, in trial 3, there was a significant ($P < 0.05$) decrease in bacterial counts in treatments 4 and 5 compared with the positive control on all storage days (Table 4-3). On day 7, only treatment 4 was lower ($P < 0.05$) than the positive control. A similar decrease in total plate counts was revealed for treatments 4 and 5 on days 14 and 28 when compared to positive control. The treatment difference can be largely attributed to the decrease in total plate count for treatment 4 and 5 when compared to the positive control on all storage days in trial 3 and days 14, 21, and 28 in trial 2. The time effect was due largely to the increase in total plate counts after 28 days when compared to day 0 in all trials. There appears to be a bacteriostatic effect. Trial 2

(Table 4-2) showed that there were no significant ($P > 0.05$) decreases on day 0. Although there was a difference of bacterial counts in the treatments, both trials illustrated that there was at least a 1 or less than 1 log difference of bacterial counts over time. The trials also showed that there was a higher decrease in bacterial counts in treatments 4 and 5 than in treatments 6 and 7, with the exception of day 14 in trial 2 and day 0 and 7 in trial 3.

Trial 1 shows higher bacterial counts compared to trials 2 and 3. Trial 1 used turkey that was purchased as thigh meat and converted to ground turkey, whereas, trials 2 and 3 used ground turkey that was already prepared prior to sample preparation. However, the bacterial counts of the raw material (treatment 1) were higher in trials 2 and 3 compared to trial 1. According to the Nationwide Raw Ground Turkey Microbiological Survey (NRGTMS) (USDA-FSIS, 1996) the initial microflora that is present on the chilled carcasses of the animal primarily comprises of gram-negative aerobic psychrotrophic bacteria. Once the meat is grinded, the surface area bacteria are distributed throughout the product. This article also shows that meats that are processed at a larger scale produce more pathogens than those that are processed in smaller quantities. The thigh meat that was used in trial 1 was grounded in a smaller batch, whereas; trials 2 and 3 were commercially grounded on a larger scale. The NRGTMS (1996) showed that out of the 296 samples that were observed, 100% of the samples tested positive for aerobic plate counts (APC). Based on the results that were obtained from this observation, raw ground turkey contains 4.15 cfu/g of APC (USDA-FSIS, 1996). Freshly processed carcasses can contain 10^3 to 10^4 cfu/cm^2 microorganisms (USDA-FSIS, 1997).

Trial 1 showed that there was a time effect for each treatment over time, a treatment effect between the different treatments, and a time*treatment interaction ($P < 0.05$). Table 4-1 shows that there was no significant difference in treatments 3 through 7 on day 0, however, there

is a 2 and 1 log increase between treatments 3 and 4 and 3 and 5, respectively. This shows that the galangal extract induce microbial effect on the treated samples compared to treatment 3. Day 7 shows that there was a significant decrease ($P < 0.05$) in treatments 6 and 7 compared to treatment 3. Treatment 3 shows bacteriostatic properties, with the exception of days 7 and 28. Treatment 4 shows that there is a significant decrease ($P < 0.05$) over time, where there is a log difference of more than 1 with the exception of day 7 and 28. The galangal extract has a bacteriostatic effect on Treatment 4 because there is a log decrease in the last two weeks compared to the first two weeks of the study, whereas, in treatment 3 no decrease was observed in the last two weeks compared to the first two. Treatment 5 shows that there is a significant difference ($P < 0.05$) in all of the days, with the exception of days 7 and 28. Treatment 6 shows no significant difference ($P > 0.05$) amongst the days. Treatment 7 shows significant decrease ($P < 0.05$) between days 7 and 14. There is also a significant decrease ($P < 0.05$) on days 21 and 28 in treatment 7. Overall, table 4-1 shows that the extract had no effect on the treatments compared to the positive control, however, the extract seemed to stabilize growth over time.

Trial 2 also showed that there was a time effect of each treatment, a treatment effect between the different treatments, and a time*treatment interaction ($P < 0.05$). Table 4-2 shows that there was no significant difference in treatments 3 through 7 on day 0 as shown in trial 1. This again shows that the extract has no effect on the treated ham samples compared to the positive control. Day 7 shows that treatments 4 and 5 were not significantly different ($P > 0.05$) to treatment 3; whereas, treatments 6 and 7 were significantly different ($P < 0.05$) to treatment 3. Treatments 6 and 7 have higher bacterial counts than treatments 4 and 5 compared to the positive control. Over time, treatments 3, 4, and 5 showed a 1 or less log decrease from day 0 to day 7; whereas, treatments 6 and 7 showed a less than 1 log increase from day 0 to 7, which could be a

result of bacteriostatic. Treatments 3 through 7 ended up with higher counts on day 28 compared to day 0 and 7. There was no significant decrease ($P < 0.05$) in treatment 3 between day 0 and 7, but an increase on days 14, 21 and 28. Treatment 4 and 7 showed no significant difference ($P > 0.05$) with the exception of an increase on day 28 in treatment 7. Treatment 5 showed that there were no significant difference ($P > 0.05$) between day 0 and 21 and day 14 and 28. However, day 7 showed a significant ($P < 0.05$) decrease from day 0 in treatment 5. Treatment 6 was significantly increase ($P < 0.05$) on days 0, 7, 14 and 28; but showed a decrease on day 21.

Trial 3 also showed that there was a time effect of each treatment, a treatment effect between the different treatments, and a time*treatment interaction ($P < 0.05$). Table 4-3 shows that there was a log or more reduction in treatments 4 through 7 compared to treatment 3 on day 0. Treatment 4 has a less ($P < 0.05$) bacterial count compared to treatment 6 on days 0, 7, 14, 21 and 28. This illustrated that the GE had an effect on treatment 4 compared to the positive control. Treatment 3 showed a significant decrease ($P < 0.05$) between day 0 and 7; but showed an increase on the bacterial counts on day 14. Treatment 5 showed a significant decrease ($P < 0.05$) on day 14 compared to days 0, 7, 21 and 28. Treatment 6 showed a significant increase ($P < 0.05$) from day 0 compared to all of the other days and that there was at least a 1 or more log increase. Treatment 7 showed a significant increase ($P < 0.05$) from day 0. Both treatments 4 and 5 seemed to show stability in bacterial counts over time compared to treatments 3, 6, and 7, which showed a bacterial increase. The galangal treatment in treatments 4 and 5 showed bacteriostatic activity compared to the other treatments.

Staphylococcus aureus Plate Count

S. aureus counts were not significantly different ($P > 0.05$) among the treatments when compared to the positive control on all storage days (Table 4-4). The data revealed no significant time and time by treatment interaction ($P > 0.05$) (Table 4-4). A significant treatment difference

was revealed which was due to the significantly lower *S. aureus* counts ($P < 0.05$) for the uncooked and cooked negative controls on all storage days when compared to all the GE treatments.

In general there was treatment difference among the treatments when compared to the negative controls. Day 0 showed no significant difference ($P > 0.05$) in *S. aureus* between the treatments with the exception of the negative control and uncooked sample (treatments 1 and 2) (Table 4-4). Treatment 4 and 5 was one log lower ($P > 0.05$) than the positive control on day 0. Treatments 4 and 5 also had lower ($P > 0.05$) counts than treatments 6 and 7 on days 0, 21 and 28. This illustrates that the method of application of the galangal extract is a major factor. The galangal extract seems to have a more effective outcome as an ingredient in the formulation of the product than as a post-treatment after cooking. Treatments 4 and 5 had a variation of bacterial counts over time, which indicates that there was less than 0.5 log variation between the days with the exception of day 14 in treatment 4. The counts in treatment 5 through 7 were higher ($P > 0.05$) than the positive control on days 7. Days 7 through 28 showed that the extract had no significant difference ($P > 0.05$) or effect on the treatments 4 through 7. However, there was a decrease in bacterial counts in treatment 4 on day 7, 14 and 28 when compared to day 0. There is also a decrease in bacterial counts ($P > 0.05$) in treatment 5 on day 14 and 28. Comparing each treatment over the 4-week period, there was no significant difference ($P > 0.05$) in the bacterial counts, which shows that there were effects on each treatment over time. The negative controls had similar ($P > 0.05$) *S. aureus* counts, which remained less than 2 log cfu/gram, through 28 days storage. This also illustrates that the bacterial count remained less than 2 log cfu/gram and the survival phase of the bacteria has been extended over the storage time (Todar, 2012).

Studies have shown that *S. aureus* does not grow well with lactic acid bacteria present (Haines and Harmon, 1973). However, the organism has the ability to grow well in its preferred environment. *S. aureus* favors a temperature of 7 - 47°C, and optimum temperature of 37°C (Marriott and Gravani, 2006).

***Listeria monocytogenes* Plate Count**

The data revealed no significant difference ($P > 0.05$) in *L. monocytogenes* counts among the trials (Table 4-5). The data also revealed significant time and treatment differences and significant time*treatment interaction. In the time by treatment effect, the counts of *L. monocytogenes* increased over the storage days. The time difference was due to the increase in *L. monocytogenes.* For treatments 3 and 6, the counts increased on day 28 when compared to day 0.

Table 4-5 reveals that there was no significant difference ($P > 0.05$) between the positive control and treatments 5 through 7 on day 0. However, treatment 4 had 1.87 log reductions compared to the positive control. Day 14, 21 and 28 also showed a 1 or more log reduction in treatments 4 and 5 compared to the positive control. Treatments 6 and 7 did not show a decrease in the bacterial counts compared to the positive control with the exception of treatment 7 on day 7, 14 and 21. Over time, treatments 3 increased less than 2 logs on day 28 compared to day 7. The other treatments also increased in bacterial counts, but not as much as the positive control. This indicates that bacterial count in treatment 3 has multiplied (Whiting and Bagi, 2002) compared to its beginning stages on day 0. The other treatments (4 through 7) that were treated with the galangal extract showed a lower ($P > 0.05$) increase due to the addition of the galangal extract compared to the positive control. Treatments 6 and 7 showed a higher ($P > 0.05$) bacterial count than treatments 4 and 5, which seems to be influenced by the method of application. The organism has the ability to survive or grow in vacuum-packaged refrigerated meats. (Glass and

Doyle, 1989), which is what influenced the bacterial count increase in treatments 3 through 7 from day 0 to day 28.

pH and Objective Color Analyses

pH

The pH values showed no significant difference ($P > 0.05$) among treatments and over time from days 0 to 28 (Table 4-6). These findings revealed that the galangal extract had no effect on the pH of the RTE turkey hams. A study conducted by Glass and Doyle (1989) on the effects of pH in processed meats inoculated with 10^5 cfu/gram of *L. monocytogenes* showed that the microorganism grew well on meats near or above pH 6.

Color L*a*b*

In general, the color values showed no significant difference ($P > 0.05$) amongst the treatments (Tables 4-7, 4-8, and 4-9). The "L*" values ranged between 40 and 50 in all of the treatments, which indicates the galangal extract had no effect on the black and whiteness of the RTE product. The "a*" value also indicated that there was no significant difference ($P > 0.05$) among the samples treated with the galangal extract when compared to the positive control. The "b*" value indicated that there was no significant difference ($P > 0.05$) in all of the treatments, with the exception of treatment 3 and 4 on day 21 when compared to the raw sample (treatment 1) and treatments 1 and 2 on day 28. There was significant difference ($P < 0.05$) in the treatments 1, 2, 4, 5 and 7 on day 0 compared to day 7, 14, 21 and 28; but not a difference ($P > 0.05$) among the positive control and treatment 6.

Table 4-1. Least square means for the interactions of treatment combined storage time for total plate count in turkey hams in trial 1

	(Log cfu/g)				
Treatments*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$3.85^{b,w}$	$4.36^{c,w}$	$5.52^{d,v}$	$6.16^{b,uv}$	$6.34^{c,u}$
2	$2.77^{b,uv}$	$3.34^{d,u}$	$1.00^{e,v}$	$1.00^{c,v}$	$1.00^{d,v}$
3	$6.66^{a,w}$	$7.89^{a,u}$	$6.94^{a,vw}$	$6.91^{ab,vw}$	$7.25^{ab,v}$
4	$8.16^{a,u}$	$8.25^{a,u}$	$6.68^{b,vw}$	$6.46^{b,w}$	$7.05^{b,v}$
5	$7.65^{a,v}$	$8.25^{a,u}$	$6.25^{c,x}$	$7.28^{a,w}$	$7.71^{a,u}$
6	$7.02^{a,u}$	$7.00^{b,u}$	$6.93^{a,u}$	$7.56^{a,u}$	$7.28^{ab,u}$
7	$6.90^{a,uv}$	$7.30^{b,u}$	$6.41^{c,v}$	$7.28^{a,u}$	$6.38^{c,v}$
SEM	0.31	0.09	0.04	0.14	0.11

$^{a-e}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-x}$ means in same row with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-2. Least square means for the interactions of treatment combined storage time for total plate count in turkey hams for trial 2

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$4.44^{b,y}$	$5.36^{bc,x}$	$7.29^{a,u}$	$6.93^{a,v}$	$6.62^{bc,w}$
2	$3.63^{c,w}$	$4.73^{d,v}$	$5.63^{e,u}$	$4.67^{d,v}$	$5.24^{e,uv}$
3	$5.86^{a,v}$	$4.86^{d,w}$	$6.83^{b,u}$	$6.96^{a,u}$	$7.12^{ab,u}$
4	$5.62^{a,u}$	$4.98^{cd,u}$	$5.77^{de,u}$	$5.59^{c,u}$	$5.59^{de,u}$
5	$5.57^{a,v}$	$4.85^{d,w}$	$6.30^{c,u}$	$5.46^{c,v}$	$6.22^{cd,u}$
6	$5.64^{a,x}$	$5.98^{a,w}$	$7.53^{a,u}$	$6.79^{a,v}$	$7.72^{a,u}$
7	$5.85^{a,v}$	$5.82^{ab,v}$	$6.17^{cd,v}$	$6.18^{b,v}$	$7.32^{a,u}$
SEM	0.09	0.08	0.08	0.10	0.12

$^{a-e}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-y}$ means in same row with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-3. Least square means for the interactions of treatment combined storage time for total plate count in turkey hams for trial 3

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$5.24^{d,v}$	$4.71^{d,w}$	$4.76^{c,w}$	$5.54^{e,v}$	$7.34^{a,u}$
2	$4.55^{e,w}$	$5.34^{c,v}$	$4.75^{c,w}$	$5.81^{d,u}$	$5.46^{c,v}$
3	$8.06^{a,u}$	$6.69^{b,w}$	$7.31^{a,v}$	$7.62^{b,v}$	$7.43^{a,v}$
4	$5.43^{d,u}$	$5.70^{c,u}$	$6.03^{b,u}$	$5.77^{d,u}$	$5.72^{bc,u}$
5	$6.64^{b,u}$	$6.52^{b,u}$	$5.47^{bc,v}$	$6.62^{c,u}$	$6.22^{b,u}$
6	$6.10^{bc,v}$	$7.22^{a,u}$	$7.45^{a,u}$	$7.97^{a,u}$	$7.24^{a,u}$
7	$5.60^{cd,x}$	$6.51^{b,w}$	$7.79^{a,uv}$	$8.00^{a,u}$	$7.31^{a,v}$
SEM	0.10	0.09	0.16	0.03	0.10

$^{a-e}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-x}$ means in same row with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-4. Least square means for the interactions of treatment combined storage time for *S. aureus* plate count in turkey hams

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$1.56^{b,u}$	$1.84^{b,u}$	$1.67^{b,u}$	$1.20^{b,u}$	$1.00^{b,u}$
2	$1.33^{b,u}$	$1.00^{b,u}$	$1.34^{b,u}$	$1.00^{b,u}$	$1.00^{b,u}$
3	$5.98^{a,u}$	$5.88^{a,u}$	$5.79^{a,u}$	$4.26^{a,u}$	$5.28^{a,u}$
4	$4.96^{a,u}$	$4.94^{a,u}$	$5.28^{a,u}$	$4.78^{a,u}$	$5.08^{a,u}$
5	$4.96^{a,u}$	$5.99^{a,u}$	$5.22^{a,u}$	$5.80^{a,u}$	$4.94^{a,u}$
6	$5.56^{a,u}$	$6.33^{a,u}$	$5.22^{a,u}$	$6.36^{a,u}$	$5.71^{a,u}$
7	$5.45^{a,u}$	$5.30^{a,u}$	$5.49^{a,u}$	$6.02^{a,u}$	$5.35^{a,u}$
SEM	0.39	0.41	0.38	0.50	0.28

$^{a-b}$ means in same column with different superscripts are significantly different ($p < 0.05$)
^{u} means in same row with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-5. Least square means for the interactions of treatment combined storage time for *L. monocytogenes* plate count in turkey hams

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$1.75^{b,u}$	$2.46^{c,u}$	$2.46^{b,u}$	$1.00^{b,u}$	$1.41^{c,u}$
2	$1.86^{b,u}$	$3.20^{bc,u}$	$1.77^{b,u}$	$1.67^{b,u}$	$1.47^{c,u}$
3	$5.53^{a,w}$	$5.82^{a,vw}$	$6.70^{a,uv}$	$6.98^{a,u}$	$7.23^{a,u}$
4	$3.66^{ab,v}$	$5.95^{a,u}$	$5.68^{a,u}$	$5.87^{a,u}$	$6.13^{ab,u}$
5	$5.37^{a,u}$	$5.72^{a,u}$	$5.23^{a,u}$	$5.80^{a,u}$	$5.90^{b,u}$
6	$5.59^{a,v}$	$5.72^{a,v}$	$6.88^{a,uv}$	$6.62^{a,uv}$	$7.06^{ab,u}$
7	$5.51^{a,uv}$	$4.93^{ab,v}$	$5.95^{a,uv}$	$5.91^{a,uv}$	$6.73^{ab,u}$
SEM	0.50	0.45	0.38	0.28	0.10

$^{a-c}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-w}$ means in same row with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-6. Least square means for the interactions of treatment combined storage time for pH in turkey hams

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	6.83	6.84	6.84	6.78	6.76
2	6.92	7.04	6.98	6.99	7.02
3	6.86	6.92	6.91	6.93	6.91
4	6.98	6.90	6.91	7.03	7.01
5	6.87	6.95	6.93	6.94	7.01
6	6.90	6.98	6.95	6.96	7.01
7	6.87	6.94	6.91	6.92	6.93
SEM	0.09	0.07	0.08	0.06	0.07

*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-7. Least square means for the interactions of treatment combined storage time for "L*" color values in turkey hams

			(Log cfu/g)		
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	49.36	41.64	39.89	43.42	43.56
2	41.33	44.66	42.08	42.66	42.33
3	47.14	41.88	41.73	43.35	42.52
4	41.17	44.52	43.87	45.67	43.53
5	42.34	44.47	43.15	42.65	41.59
6	41.99	42.07	42.77	42.92	42.00
7	41.05	43.47	44.88	43.42	42.85
SEM	2.74	1.63	1.82	1.22	1.15

*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-8. Least square means for the interactions of treatment combined storage time for "a*" color values in turkey hams

			(Log cfu/g)		
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$15.11^{a,u}$	$8.63^{a,v}$	$8.57^{a,v}$	$8.70^{a,v}$	$8.78^{a,v}$
2	$7.43^{b,u}$	$8.96^{a,u}$	$8.16^{a,u}$	$11.12^{a,u}$	$8.02^{a,u}$
3	$8.46^{b,u}$	$9.00^{a,u}$	$10.53^{a,u}$	$9.03^{a,u}$	$10.04^{a,u}$
4	$6.88^{b,u}$	$10.22^{a,u}$	$9.38^{a,u}$	$9.09^{a,u}$	$8.26^{a,u}$
5	$6.90^{b,u}$	$8.10^{a,u}$	$8.74^{a,u}$	$9.30^{a,u}$	$8.72^{a,u}$
6	$7.40^{b,u}$	$7.89^{a,u}$	$8.24^{a,u}$	$9.49^{a,u}$	$9.40^{a,u}$
7	$7.68^{b,u}$	$7.50^{a,u}$	$7.01^{a,u}$	$7.91^{a,u}$	$8.40^{a,u}$
SEM	1.06	0.77	1.37	1.07	0.95

$^{a-b}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-v}$ means in same column with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

Table 4-9. Least square means for the interactions of treatment combined storage time for "B" color values in turkey hams

	(Log cfu/g)				
Treatment*	Day 0	Day 7	Day 14	Day 21	Day 28
1	$14.29^{a,u}$	$7.57^{a,v}$	$6.82^{a,v}$	$8.09^{a,v}$	$7.80^{a,v}$
2	9.04^{a}	$5.74^{a,uv}$	$4.24^{a,v}$	$6.42^{ab,uv}$	$4.16^{b,v}$
3	$11.52^{a,u}$	$5.06^{a,u}$	$5.72^{a,u}$	$5.34^{b,u}$	$5.60^{ab,u}$
4	$8.83^{a,u}$	$6.23^{a,v}$	$5.57^{a,v}$	$5.40^{b,v}$	$5.07^{ab,v}$
5	$9.80^{a,u}$	$6.01^{a,v}$	$6.57^{a,v}$	$6.43^{ab,v}$	$6.20^{ab,v}$
6	$9.39^{a,u}$	$5.21^{a,u}$	$5.05^{a,u}$	$5.70^{ab,u}$	$5.83^{ab,u}$
7	$9.62^{a,u}$	$6.08^{a,v}$	$5.38^{a,v}$	$6.01^{ab,v}$	$6.84^{ab,uv}$
SEM	1.15	0.62	0.90	0.44	0.59

$^{a-b}$ means in same column with different superscripts are significantly different ($p < 0.05$)
$^{u-v}$ means in same column with different superscripts are significantly different ($p < 0.05$)
*1=Raw meat (Negative Control), 2=No GE + cook, 3=Cooked meat, no GE + Inoculum (Positive Control), 4=0.5% GE + Inoculum, 5=1.0% GE + Inoculum, 6=0.5% GE (Post-Treatment), 7=1.0% GE + Inoculum (Post Inoculum)
SEM = Standard Error Mean

CHAPTER 5
SUMMARY AND CONCLUSION

The objective of this study was to observe the effects of galangal extract in RTE turkey hams against *L. monocytogenes* and *S. aureus*. The study has shown that there was no significant ($P > 0.05$) effect against both organisms. However, treatment 4 (0.5% GE prior to cooking) showed stability in the bacterial counts over time. In general, turkey samples treated with GE prior to cooking resulted in 1 log cfu/gram reduction in ($P > 0.05$) *S. aureus* on day 0 and 1 log cfu/gram reductions ($P > 0.05$) in *L. monocytogenes* on days 0 (0.5% GE only), 14, 21 and 28 when compared to the positive control. Although the mechanisms of the GE have not yet been investigated, the antimicrobial effects of the extract seem to be more effective as an ingredient incorporated into the meat mixture when added prior to cooking as compared to applying the GE on the surface of the cooked meat (post processing). Other studies have shown that herbs that are extracted in ethanol have bactericidal activity against *S. aureus* and *L. monocytogenes* (Hsu et al., 2010; Oonmetta-aree et al., 2006; Weerakkody et al., 2010, Weerakkody et al., 2011), however, these studies did not factor in the composition of RTE meats seeing that the disc diffusion method was used instead. This study was used as a model to determine the best application method for the GE.

The 1.0% maximum usage level of GE was insufficient to achieve at least 2 log reductions in *L. monocytogenes* and *S. aureus* in this study. In future studies the amount of GE should be increased from 1.0% to at least 5% (in 1% increments) in order to achieve antimicrobial reductions in *L. monocytogenes* and *S. aureus* greater than 1.0 log. The goal is to achieve 5 log reductions while maintaining the inherent quality characteristics of the turkey ham product. Cheah and Hasim (2000) used up to 10% galangal extract, produced from the galangal plant rhizome in cooked minced beef for antioxidant and antimicrobial properties. The

researchers achieved a reduction in total aerobic bacteria of 0.71 log cfu/gram. Galangal extract was produced from the galangal flower in our study. Future studies should also focus on bacterial count reductions during days 0, 7 and 14, which are the days when RTE meats are primarily consumed (MAFF, 2000).

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BIOGRAPHICAL SKETCH

Melissa Cadet was born in Miami, Florida in 1986. In 2010, she was awarded a Bachelor of Science degree from the College of Engineering Sciences Technology and Agriculture at Florida Agricultural and Mechanical University in Tallahassee, Florida. She was awarded the Florida Agricultural and Mechanical University Feeder Fellowship at the University of Florida Graduate School Office of Graduate Minority Programs, to study for the Master of Science degree in the College of Agricultural and Life Sciences, in the Department of Animal Sciences, under the supervision of Dr. Sally K. Williams. Melissa will receive a Master of Science degree in August 2012.