EFFECT OF A CARRAGEENAN/CHITOSAN COATING WITH ALLYL ISOTHIOCYANATE ON MICROBIAL LOAD, SPOILAGE, AND QUALITY PARAMETERS IN CHICKEN BREASTS.

by

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(Under the Direction of Manpreet Singh)

ABSTRACT

Edible antimicrobial coatings have recently emerged as a novel approach to increase the shelf life of products. An edible antimicrobial coating consisting of Chitosan/Carrageenan with the antimicrobial Allyl Isothiocyanate (AITC) at varying concentrations was found to be inhibitory against *Campylobacter. coli* and *S.* Typhimurium at low and high inoculum concentration on chicken breasts over a 21-day storage period at 4°C ($p \leq 0.05$). Additionally, the natural microflora of chicken breasts such as Lactic Acid Bacteria, Aerobic Bacteria, Yeasts, and Molds was also inhibited by the antimicrobial coating over a 21-day storage period at 4°C ($p \leq 0.05$). However, the antimicrobial coating had a detrimental effect on the quality of the chicken breasts by decreasing the pH, increasing rancidity, and developing undesirable colors. Edible antimicrobial coatings reduce *Campylobacter. coli, S.* Typhimurium, and spoilage bacteria populations, thus showing potential to be used as antimicrobial packaging to increase shelf life of fresh poultry.

INDEX WORDS: Antimicrobial Coating, Carrageenan/Chitosan, Allyl Isothiocyanate, Chicken Breast

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CHAPTER 1

INTRODUCTION

Over the past 40 years, the poultry industry has moved away from whole chicken sales and concentrated on selling chicken parts (Aho, 2021). However, chicken parts are more susceptible to adulteration due to contamination during further processing. Therefore, edible antimicrobial coatings have arisen as possible solutions. Edible antimicrobial coatings are thin layers prepared from naturally occurring polymers and applied on a food surface by different mechanisms such as spraying, brushing, and dipping. These edible coatings may also have active components such as organic acids, bacteriocins, proteins, herbs, and spices. These coatings are applied after further processing and release antimicrobials throughout the storage period, making them ideal for further process meats. However, they are disadvantages; the low pH of the coating may alter the quality of the chicken breasts. Additionally, the antimicrobials added to the coatings may also cause changes in odor, color, pH, and rancidity.

Previous studies have developed methods to create a Chitosan/Carrageenan coating with AITC. Chitosan is used because as a cationic polysaccharide obtained by deacetylation of chitin; it forms an excellent film with oxygen barrier properties, intrinsic antimicrobial activity, and antioxidant activity (Xia et al., 2011). Additionally, Carrageenan is a sulfated anionic polysaccharide extracted from red seaweed and has already been used extensively in the food industry as a gelling, stabilizing agent, and has excellent film-forming properties (Olaimat et al. 2015). This experiment incorporated Carrageenan and Chitosan using a layer-by-layer technique creating a coating in which an antimicrobial can attach and diffuse through. The antimicrobial used was Allyl Isothiocyanate (AITC) extracted from mustard. AITC has shown to be inhibitory towards pathogenic bacteria and a potent anticancer agent.

The objective of the experiment was to observe if the edible antimicrobial coating would reduce *Salmonella* Typhimurium, *Campylobacter coli,* lactic acid bacteria, aerobic bacteria, yeasts, and molds on chicken breasts over a 21-day storage period at 4° C. The study also aimed to evaluate the effect of the antimicrobial coating on pH, rancidity, and color over the storage period. Additionally, the antimicrobial resistance of *Salmonella* typhimurium and *Campylobacter. coli* to AITC over the storage period was also evaluated.

REFERENCES

Aho, P. (2012). *US Boneless, Skinless, Chicken Breast Industry Continues to Grow*. FeedStrategy. https://www.feedstrategy.com/uncategorized/us-boneless-skinless-chicken-breast-industry-continues-to-grow/

Xia, W., Liu, P., Zhang, J., & Chen, J. (2011). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids, 25*, 170-179.

Olaimat, A. N., & Holler, R. A. (2015). Control of *Salmonella* on fresh chicken breasts by k-carrageenan/chitosan-based coatings containing allyl isothiocyanate or deodorized oriental mustard extract plus EDTA. *Food Microbiology, 48*, 83-88. https://doi.org/10.1016/j.fm.2014.11.019

CHAPTER 2

LITERATURE REVIEW

POULTRY INDUSTRY TRENDS

Global Poultry Production

Poultry refers to the edible flesh, with adhering bones, of any bird commonly used as food and includes chickens, ducks, geese, turkey, quail, and pheasant (Britannica, 2020). In 2019, the global poultry market increased by 6% to 231.5 billion USA dollars, rising for the third year in a row (Berkhout, 2020). The top three countries with the highest volume of poultry consumption, are China (20 million tons), the United States (19 million tons), and Brazil (12 million tons) (Berkhout, 2020). The countries with the highest average poultry consumption per capita in 2019 were Malaysia (63 kg per person), the United States (58 kg per person), and Brazil (57 kg per person) (Berkhout, 2020).

Global poultry production worldwide increased to 130 million tons in 2019, and the countries with the highest volumes of poultry production in 2019 were the United States (23 million tons), China (20 million tons), and Brazil (16 million tons) (USDA, 2020). The total export volume of poultry has increased at an annual average rate of 3.3% over 2009-2019 (USDA, 2020). In 2019 overseas shipments of poultry increased by 2.2% to 17 million tons. The leading poultry exporters were Brazil and the United States, which accounted for 24 and 22% of total exports, respectively (Berkhout, 2020). The Netherlands, Poland, Belgium, Turkey, and Germany were the following top exporters. The average poultry price was 1,644 US dollars per ton in 2019, and the highest cost was in Thailand at 2,683 US dollars per ton, while the lowest price was in the United States at 1,045 US dollars per ton (Berkhout, 2020).

The Food and Agriculture Organization (FAO) estimated that global poultry meat production would reach 137 million tons in 2020. However, in 2020 the COVID-19 pandemic affected several poultry markets such as in India, the covid lockdown caused the unavailability of the workforce and therefore led to a decrease in consumer demand, (ii) in Thailand, the reduction in restaurants and street markets decreased poultry consumption, and (iii) in the United States, the declining food and restaurant sales compounded by labor shortages led to reduced poultry consumption (Berkhout, 2020). Additionally, poultry facilities have had to halt many expansion projects due to new covid restrictions that require distancing in workspaces and additional sanitary practices.

Poultry Production in the United States

The USA is the world's top poultry producer at the lowest prices due to its approximately 30 federally inspected companies (USDA, 2020). These federally inspected poultry facilities operate as a "vertically integrated" entity, meaning that the farmers do not own the broilers; they own the land and supply labor, housing, equipment, utilities, and litter. The companies own the broilers and are responsible for veterinary services, medication, and fuel for heating the houses. Growers are paid according to the number of pounds of live birds delivered to the processing plant and are given incentives for livability, feed efficiency, and minimal condemnation (Cunning, 1996 and 1997). About 25,000 family-owned farms have contracts with these companies and produce about 95% of the broilers for consumption (USDA, 2020). The remaining 5% of the broilers are produced at farms owned by the top 30 companies. In the USA, in 2020, poultry facilities produced almost 59.75 billion pounds of chicken, of which more than 45 billion pounds of chicken products were marketed as ready-to-cook (USDA, 2020). A growing percentage of the US poultry industry revenue comes from the export of poultry products deemed undesirable for USA consumers, such

as dark meat and paws (Owens et al., 2009). The top producers of broilers were Georgia, Arkansas, North Carolina, Alabama, and Mississippi.

In Georgia, eggs and broilers are the two largest agricultural commodities, making up nearly 40 percent of the state's production value (UGA, 2020). In the US, the per capita consumption of broilers has doubled since 1978; however, the production of broilers in Georgia has almost tripled in that time. Georgia is responsible for more than 15% of the broiler meat produced in the USA (UGA, 2020). Additionally, the combined export value of chickens and turkeys was 5.53 billion USA dollars in 2013. The factors that contribute to Georgia's poultry market are the efficiency of domestic production, income, and population growth of the domestic market, shifts in exchange rates, trade policy, and relatively low prices compared to other markets (Davis et al., 2019). The state is also strategically located for exports due to the port of Savannah, which moves 32 percent of the total USA waterborne poultry export (Davis et al., 2019). In total, according to the University of Georgia, the annual economic impact of the poultry industry in the state is 28 billion USA dollars and 100,000 jobs a year. On average, Georgia produces 29 million pounds of chicken, 6.3 million table eggs, and 5.5 million hatching eggs (GPI, 2021).

Chicken Breast Production

Over the past 40 years, the poultry industry has shifted from whole carcass chicken sales and concentrated on selling chicken parts/cuts (Aho, 2021). The top 3 chicken cuts frequently purchased in supermarkets are skinless chicken breast value packs, drumsticks and thighs. One chicken yields two drumsticks, two thighs, two wings, and one breast split in half across the rib bone (BCcampus, 2018).

In the 1980s, chickens were processed and sold as whole bone-in chicken; however, in the past forty years, the poultry industry has transformed into the boneless skinless breast industry.

The industry changed rapidly from a few hundred million pounds in 1985 to over 6 billion pounds of skinless chicken breast in 2010 because of increased consumer demand and convenience (Aho, 2021). Poultry producers, farmers, and veterinarians became increasingly better at producing chicken breast by raising fast-growing chickens with large breasts. Veterinarians have kept the birds healthy, and nutritionists have fine-tuned the birds' diet. Processors have also been able to lower the cost of producing chicken breast. The price fell dramatically from 4.50 USA dollars in the 1980s to 1.25 USA dollars in 2010 (Aho, 2021). A conservative estimate is that by 2030, 235 million people in the USA will be eating 30 pounds of boneless skinless chicken breast, requiring the production of at least 10 billion pounds broilers (Aho, 2021). These radical changes were due to the changes in modern chicken production including new poultry processing line speeds of 70 to 140 birds/minute (Moran, 1999). The following section discusses the details of poultry processing.

POULTRY PROCESSING

Pre-Slaughter

Broilers are typically reared on litter in enclosed houses with approximately 20,000-25,000 broilers per house (Owens, 2009). Most broilers are processed when they reach 6-8 weeks of age and weigh about 4-8 lbs.

Poultry must be "harvested" before it can be processed. First, the broilers must go through a withdrawal of food and water 8 to 12 h before harvest. Removing the feed and water reduces the incidence of fecal carcass contamination during processing (Benoff, 1996; Northcutt et al., 1997; Bilgili, 2008). The farmworkers or a mechanical harvester must pick up the chicken from the house, transport them to a container (coops, crates, or cages) and transport them to a processing plant in cages for no more than two hours (ideally). The cages vary in size and the number of

compartments per unit, but they can be 2, 4, 5, or 6 compartments with 15-25 broilers (Bennett, 2008). When the broilers arrive at the processing plant, they are held, unloaded, and shackled.

At this point, an inspector observes the birds looking for signs of abnormal conditions such as swelling/edema of facial tissues, respiratory distress, off-colored diarrhea, lameness, skin lesions, etc. (Owens, 2009). Broilers that are diseased when they arrive at the processing facility are immediately condemned and disposed of adequately.

First Processing

Stunning and Bleeding

After the birds are unloaded and shackled on a conveyer belt in a dark room, they are stunned. Stunning is the first step to render the bird unconscious before slaughter, and several studies have shown that stunning is humane and provides quality improvements. Stunning is done by submerging the bird's head in a solution of 1% NaCl and passing an electric current of 10-25 volts through it. When correctly done, the bird is stunned for 60-90 sec (Fletcher, 1993).

After stunning, the shackles move the birds to the cutting machine for exsanguination. The cutting machine uses a rotating blade to cut the jugular veins and carotid arteries on both sides of the neck (Fletcher, 1993). This process happens 7 to 10 sec after stunning, followed by a 2-3 min bleed out, the bird loses about 30 to 50 percent of its blood and eventually loses brain function and dies (Singh et al., 1984).

Scalding and Defeathering

After the stunning and bleed-out stage, the birds enter the scalder, where the feathers are loosened by submerging the carcasses in hot water. Scalding can occur at 53°C for 120 sec (known as soft scalding) or 62°C for 45 sec (known as hard scalding) (Owens, 2009). Scalding may happen

at multiple temperatures during several stages, and some poultry facilities have 1-3 scalders in temperature ranges of 48°C to 62°C for up to 4 min (Owens, 2009).

After scalding, the feathers are picked by the picker (picking machine). The picker consists of rows of a rotating cluster of flexible, ribbed, rubber fingers that rapidly rotate, pulling off the feathers. The last step in the picking process is to burn off the hairlike filoplume structure on the skin because they are undesirable for the consumers. The head and feet are removed from the carcass, and the carcass continues onto evisceration (Owens, 2009).

Evisceration and Inspection

Evisceration is the removal of edible and inedible viscera from the carcasses. The techniques vary from one processing plant to another, but the general goals are to open the body cavity by cutting the posterior tip of the breastbone to the cloaca, removing the viscera, which includes the gastrointestinal tract, reproductive organs, lungs, and finally, the edible viscera or "giblets," which consists of the heart, liver, and gizzard. The giblets are harvested, trimmed, washed, and saved (Owens, 2009). After the evisceration process, the bird is inspected.

Poultry inspection regulations require every single bird to be inspected. The Food Safety and Inspection Service (FSIS) inspector examines the internal/external surfaces of the chicken and the internal organs for any sign of diseases or contamination (Owens, 2009). When the birds pass the inspection, they go through an inside-outside bird washer (IOBW) and are subsequently chilled.

Chilling

The primary objective of chilling poultry is to reduce microbial growth to ensure food safety and extend storage periods. US regulations require that a temperature of 4°C be achieved no more than 4 hours after death (USDA, 2020). In the US, the most common method of chilling

poultry is water chilling. Water chilling usually involved multiple stages of tanks. The first stage (pre-chiller) is from 7°C to 12°C and lasts for 10 to 15 sec (Veerkamp, 1989). The purpose of pre-chilling is to reduce the carcass temperature to prevent quality deterioration in the second stage (chilling), the birds are in a bigger tank (4°C) in the entrance and (1°C) in the exit, and this chilling cycle lasts 45 to 110 min and may contain antimicrobials such as chlorine and peroxyacetic acid (Veerkamp, 1989). Lastly, poultry processors have added a finishing chiller to provide a final rinse with antimicrobials at a higher concentration.

Further Processing

Second Processing

After first processing, carcasses are cut into parts, carcasses can be cut into many configurations. Some examples are half carcasses, leg quarters, wings, and breasts (Owens, 2009). The chickens are commonly cut into two halves, four quarters, eight parts (breast, wings, thighs, drumsticks), or nine parts (includes a keel piece). The immense demand for boneless breast has created a high-priced market and a high degree of customer demand for quality. Chicken breasts are difficult to produce because they have no skin to hide defects, blemish, or retain water.

Aging

Aging or maturing has become common in the meat industry, with reports suggesting that if the meat was deboned too soon after slaughtering, it becomes tough (Fremery, 1960). Modern processing plants age the meat at refrigeration temperatures for 2-4 hours after slaughter to reduce costs and water loss. The aged meat is deboned and cut into fillets (Fremery, 1960). Processors either hand deboned or use machines. After the carcass is cut into parts, machines sort the parts by weight to ensure uniformity. The chicken breasts can also be further cut into fillets mechanically or by hand.

Packaging

After going through second processing, the poultry products (whole carcasses and parts) are packaged. Packaging is constantly changing to maintain and/or improve the quality of poultry products. There is limited research being conducted on film permeability, vacuum, and modified atmosphere packaging, including parameters such as oxygen scavengers, moisture absorbers, temperature-compensating films, and antimicrobial packaging. Antimicrobial packaging is further explored in a forthcoming chapter.

POULTRY PATHOGENS, SPOILAGE, AND QUALITY

As previously mentioned, the FSIS oversees inspections in processing plants to prevent the presence of foodborne pathogens. In 2019, the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) estimated that 48 million people get sick, 128,000 are hospitalized, and 3,000 people died due to foodborne diseases each year in the US (CDC, 2019). The number and types of microorganisms depend on the animal's physiological status, the sanitary condition of the meat source, slaughtering/handling circumstances, the microbial load of ingredients, and storage/distribution conditions. The most common bacteria found in poultry are mesophilic aerobes, *Enterobacteriaceae*, psychrotrophic, E.coli, *C. perfringens*, *S*. aureus, *Salmonella*, *C. jejuni* and *L. monocyte-genes*. The bacteria evaluated in this research will be furthered explored in this section.

Salmonella Typhimurium

Characteristics

Salmonella enterica serotype typhi is responsible for typhoid fever and has been a burden to many countries over generations (Barnett, 2016). The organism was first discovered in 1980 by the German pathologist Karl Eberth. Georg Gaffky first cultured it, and several years later

Alrmorth Writh developed a vaccine for the disease (Barnett, 2016). *Salmonella enterica* serotype typhi is a gram-negative, rod-shaped, flagellated bacterium (Crump et al. 2015 and Perry et al. 2002). *Salmonella* is a non-spore-forming bacteria with cell diameters between 0.7-1.5 µm and length between 2-5 µm (Fabrega, 2013). *Salmonella* is a facultative anaerobe that grows between 8 °C and 45 °C and at pH ranges between 4-8 (Feasey, 2012).

Taxonomy

The genus *Salmonella* is part of the family Enterobacteriaceae. The genus comprises two species, *S. bongori* and *S. enterica,* and the latter is divided into six subspecies: I, *S. enterica* subsp. *enterica;* II, *S. enterica* subsp. *salamae;* III, *S. enterica* subsp. *arizonae;* IV, *S. enterica* subsp. *diarizonae;* V, *S. enterica* subsp. *houtenae;* and VI, *S. enterica* subsp. *indica* (Brenner, 2000 and Gillespie, 2006). The group can be further divided into more than 2500 serotypes. The serotypes are defined based on somatic O and flagellar H antigens. *Salmonella* Typhimurium is a serotype of subspecies I. The full name of *S.* Typhimurium is *Salmonella. enterica* subsp. *enterica* ser. Typhimurium.

Pathogenesis Model

S. Typhimurium infections begin with the ingestion of contaminated food or water. When *Salmonella* enters the stomach, it protects itself against severe acid shock by activating the acid tolerance response (ATR), which induces a pH-homeostatic function to maintain the intracellular pH higher than the extracellular environment (Foster et al., 1991). After entering the small vowel, the salmonellae go through the intestinal mucus layer and attach to intestinal epithelial cells. Shortly after adhesion, the salmonellae signal the epithelial cells to modify their cytoskeleton and create a vacuole (SCV) that engulfs the salmonellae. After the vacuole is engulfed, the epithelial brush border is reconstructed (Santos et al., 2003). To avoid being fussed with secondary

lysosomes, *Salmonella* changes the endocytic and trafficking functions in the host cell. Then the SCV moves towards the Golgi Apparatus to obtain nutrients and membrane fragments; this event is essential for salmonellae replication (Garcia, 1995 and Rathman, 1997). In addition, *Salmonella* induces the formation of long filamentous membrane structures called *Salmonella*-induced filaments (SIFs) (Garcia, 1995 and Rathman, 1997). It is unclear why SIFs are formed, but it is speculated that they increase the availability of nutrients. Lastly, the salmonellae are disseminated through the lymph and bloodstream.

Virulence Factors

To overcome the host defense mechanisms *S.* Typhimurium, possess several virulence factors. Most of the genes encoding for virulence factors are located within *Salmonella* pathogenicity islands (SPIs), virulence plasmid (pSLT), or chromosomes. They are five SPIs (SPI-1 to SPI-5) that are involved in *S.* typhimurium virulence, together with plasmids, adhesins, flagella, and other essential biofilm components (Marcus et al. 2000 and Coburn et al. 1999). SPIs aid the invasion of epithelial cells by rearranging the cytoskeletal actin (Fabrega et al., 2013). Virulence plasmids have antimicrobial resistance genes that aid in host infection. Adhesins aid in several processes such as intestinal fluid accumulation, biofilm formation, and epithelial cell adhesion (Fabrega et al., 2013).

Human Infection

S. Typhimurium passes through the lymphatic system of the intestine into the blood of the patients and is carried to various organs (liver, spleen, kidneys) to form secondary foci. When *Salmonella* infects humans, it required 12-72 hours of incubation. Exotoxins cause fever, vomiting, and diarrhea for 2-7 days (Feasey, 2012). In severe cases, the loss of electrolytes causes hypovolemic shock, and septic shock may also develop.

Campylobacter. coli

Characteristics

C. coli in humans causes campylobacteriosis which is the most frequent diarrheal disease reported in the United States, with an estimated 1.5 million cases per year (CDC, 2019). *Campylobacter* was first observed in stool samples in 1886 by Theodor Escherich but was not implicated as a cause for human diarrhea until 1957 (Kaakoush, 2015). *C. coli* is a Gram-negative, microaerophilic, non-endospore forming bacteria from the genus *Campylobacter* (Prescott, 2005). *Campylobacter* species vary from spiral to rod or curved shape depending on the species. *C. coli* specifically has an S-shape and can move via unipolar or bipolar flagella (Vadamme et al., 2006). *Campylobacter* grows between 37-42°C in a microaerophilic environment (CDC, 2019).

Taxonomy

Campylobacter contains 16 species and six subspecies. The species *Campylobacter lari, Campylobacter upsaliensis, Campylobacter leveticus, Campylobacter coli* and subspecies *Campylobacter jejuni* subsp. *jejuni* and *Campylobacter jejuni* subsp. *doylei* are the most commonly isolated from human and animal diarrhea. (Friedman et al., 2000; Gulliespies et al., 2003; Taboada et al., 2013).

Pathogenesis

C. coli are thought to mainly be transmitted via handling and eating of raw or undercooked food products. However, due to their large natural reservoir, they can also be transmitted via soil and water (Nilsson et al. 2017). The infections dose range between 1000-10000 colony forming units (CFU) but concentrations as low as 500-800 CFU can be infectious (Humphrey et al. 2007). First, the organism penetrates the gastrointestinal mucus by using its high mobility and spiral shape. Then the bacterium attaches to the gut enterocytes and releases toxins such as enterotoxins

and cytotoxins. The adhesion to eukaryotic cells is mediated by several proteins, including *Campylobacter* adhesion to fibronectin proteins (CadF), which binds specifically to fibronectin in the cell membrane (Monteville et al. 2003). The toxins released by the microorganism correlate to the severity of the disease. Recent research has tried to explain how *Campylobacter* survives in aerobic conditions. Some studies have suggested aerotolerance, nutritional/metabolic adaptations, viable but noncultural state, microbial commensalism, and biofilm formation (Oh et al., 2017; Haddad et al., 2009; Magajna et al., 2015; Joshua et al., 2006).

Virulence Factors

Campylobacteriosis seems to be dependent on several virulence factors involving adhesion, invasion, and bacteria motility. *Campylobacter* causes cell death by releasing AB toxins composed of three subunits encoded by *cdtA, cdtB,* and *ctdC.* These toxins have DNAase activity, which causes the DNA double-strand breaks during the growth phase of the cell cycle (Whitehouse et al. 1998).

Campylobacteriosis symptoms

The onset of disease symptoms occurs 2 to 5 days after infection but can range from 1 to 10 days. The most common clinical symptoms of *Campylobacter* infections include diarrhea, abdominal pain, fever, headache, nausea, and/or vomiting; symptoms can last 3 to 6 days (WHO, 2020). Death is uncommon and is usually confined to immunocompromised patients. Other complications such as hepatitis, bacteremia, pancreatitis, and miscarriages have been reported. Patients have also developed Guillain-Barre syndrome a polio like paralysis that causes neurological symptoms (WHO, 2020).

S. Typhimurium and C. coli Incidence in the Poultry Industry

Salmonella Typhimurium and *C. coli* have multiple routes of entry in the poultry production system. The first route of entry occurs during the pre-slaughter. *Salmonella* can colonize the reproductive areas such as the ovaries and oviduct and be vertically transmitted into the eggs (Gast et al., 2007). When the chicks hatch, if they are contaminated, they are more likely to have chronic problems due to their lack of microbial diversity and unstable gut microbiome (Oakley et al., 2014). There is no evidence of *C. coli* being vertically transmitted. However, *Salmonella* Typhimurium and *C. coli* can also contaminate poultry through horizontal transmission across many reservoirs, such as cattle, small ruminants, and pigs in the farms. *Campylobacter* can also survive in the feather follicles and pores of birds (Chantarapanont et al., 2003). During processing, cross-contamination may occur during scalding, picking, evisceration, and chilling. During scalding, *Salmonella* Typhimurium and *C. coli* can be attached to the skin, avoiding antimicrobials, and acting as a source of infections in subsequent stages (Kim et al., 1996). Picking can cause peristaltic movements, leading to the expulsion of feces (Berrang et al., 2001). Furthermore, since the rubber fingers are not changing between carcasses, there is a high likelihood of cross contaminations (Nde et al., 2007). A faulty evisceration can lead to contamination of carcasses with fecal material and intestinal contents. Proper feed withdrawal is crucial to prevent contamination during this stage. The possibility of cross-contamination is high in chillers (37%) compared to other steps in processing (10-20%).

Methods of Controlling S. Typhimurium and C. coli

To prevent contamination during pre-slaughter multiple mechanisms have been developed, such as synthetic antibiotics, plant extracts, herbs, probiotics, prebiotics, and organic acids (Remington, 2017). Probiotics are more commonly used than other alternatives (Sahin, 2015). The probiotics hasten the histological and immune maturation of the intestinal tract. However,

probiotics to prevent *Campylobacter* have primarily failed because *Campylobacter* does not compete for resources with probiotic bacteria; instead, it depends on the gut microbiota to create the low oxygen environment and secondary metabolites it needs to survive (Remington, 2017). Herbs, spices, and plant extracts are also added to the feed. The mechanism of action of these botanicals is not fully understood. Some weight gains have been reported using botanical, but the results and mechanism of action are not conclusive (Diaz-Sanchez, 2015). Vaccinations were shown to be efficient and were the primary cause of the reduction of *Salmonella* incidence worldwide, especially in laying hens. An early study conducted by Stern et al. (1990) showed that inoculated chicks with *Campylobacter*-specific immunoglobins increased the colonization dose needed up to 50%. This study suggests that immune intervention and vaccination can reduce the incidence of *Campylobacter*. However, vaccines remain ineffective in reducing the incidence of *Campylobacter* in broilers. During processing, *Salmonella* Typhimurium and *C. coli* are reduced during scalding and chilling. On-line spray washing with organic acids in numerous sites in the processing line reduce *Salmonella* by 56% (Zaki et al., 2015). During chilling, the chickens are submerged in antimicrobials such as chlorine and peroxyacetic acid at multiple stages of tanks with varying concentrations. After further processing, they are few methods of controlling *Salmonella* Typhimurium and *C. coli*. Some methods being explored include modified atmosphere packaging, edible antimicrobial coatings, and oxygen scavengers.

Spoilage Bacteria

Spoilage is defined as the changes in the meat that render the products unacceptable for human consumption. It is estimated that between 3.7 and 4.2% of poultry meat is lost due to spoilage each year (Buzby et al., 2009). Spoilage of meat may be due to prolonged storage time, improper storage temperature, contamination, or high ph. Food spoilage produces physical,

chemical, or biological changes that produce off-flavors, off-colors, off-odors, and microbial growth (Remington, 2017). The shelf life of fresh poultry is approximately 4-10 days when stored at refrigerator temperatures under normal atmospheric conditions (Marenzi, 1986). The higher initial population of contamination of the freshly processed chicken will decrease the shelf-life of the product.

Lactic Acid Bacteria

Lactic acid bacteria are either rod-shaped (bacilli) or spherical (cocci) and can survive in low pH environments. Additionally, LAB, are gram-positive, low-GC, and generally nonsporulating bacteria (Sonomoto et al. 2011). LAB's ability to survive in low pH environments helps them outcompete other bacteria in natural fermentation. LAB produces lactic acid as the primary product of the fermentation of carbohydrates (Hatti-Kaul, 2018). The genera that comprise LAB are *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus,* and *Streptococcus.* Other peripheral LAB bacteria include *Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Tangenococcus, Vagococcus,* and *Weisella.* All of which are Lactobacillales order and Firmicutes phylum (Saez-Lara et al. 2015) Poultry, is particularly susceptible to Lactic Acid Bacteria due to its high water activity (a_w) and low pH. Lactic acid bacteria can cause greening of meat, off-flavors, off-odors, pH decrease, milky exudates, and slimy texture (Jay, 2005).

Aerobic Bacteria

Aerobic bacteria are organisms that can survive and grow in an oxygenated environment (Hentges, 1996). Aerobic respiration yields more energy than anaerobic respiration. These organisms are believed to be 101.5 million years old and can survive in a variety of environments. Aerobic bacteria relevant to meat is divided into three groups according to the temperature range in which they grow: mesophiles 10-45°C, psychrophiles 0-28°C, and psychographs 10-45°C, or

slow growth at 0-10°C. Psychrotrophs of which Pseudomonas is the most important and can grow at refrigerator temperature (FAO, 2020). Other aerobic organisms of interest in poultry include *Brochotrix*. *thermosphacta*, *Pseudomonas*. *fluorescens, Pseudomonas*. *fragi, Serratia*. *liquefaciens,* and *Shewanella*. *putrefaciens* (Russell, 2008; Hinton et al., 2004; Zhang et al., 2012). Aerobic bacteria can cause byproducts that change the meat's color, gas production, odor, fat decomposition, and create surface slime (Lawrie et al. 2006).

Yeast and Mold

Yeast and mold encompass a variety of microorganisms that might affect the meat. Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. Molds are fungus that grows in the form of multi-cellular filaments called hyphae (Moore, 2011). Some species can grow over a wide pH range, tolerate extreme temperature 0-47°C, and can grow in environments with low water activity ≤ 0.65 (a_w) (APHA, 1992). Additionally, yeast and molds can survive meat processes such as irradiation, high hydrostatic pressure technology, and organic acids treatments. The most common yeast and mold found in poultry are *Zygosaccharomyces* and *Aspergillus,* respectively. During their growth, yeasts metabolize some food components and produce metabolic end products which cause physical and chemical changes in food (Fleet et al. 2001). Yeast and mold contamination can lead to visible patches of mold. However, in many instances, spoilage is not visible and can lead to changes in smell, flavors, and taste (APHA, 1992).

Quality

Meat is considered a high-quality product by consumers. Meat quality is determined by (1) ingredients (all-included components), (2) nutritive-physiological aspects, (3) hygienic and toxicological aspects (microbial load/spoilage bacteria), (4) physical aspects (color, texture, pH), and (5) sensory attributes (flavor/rancidity) (Gashorn et al., 2006).

Color

Color is an essential sensory attribute, as this is one of the first quality parameters exposed to consumers. The bird's diet and genotype might influence the color. Feeding the birds corn and alfalfa can cause the skin to turn yellow due to the high lutein and zeaxanthin (EFSA, 2009). Poultry meat is referred to as "white" meat, further divided into two types of meat colors: white and dark. Poultry breast consists mainly of white muscle fibers, whereas thighs and wings are made of intermediate muscle fibers (Remignon et al., 1995). Meat color depends primarily on the contents of deoxymoyglobin (Mb), oxymyoglobin (MbO2), and metmyoglobin (MetMb) (Lebret et al., 2015). Oxidation of the purple-red Mb results in the formation of brown MetMb, and redness is measured by photometry, where the a* value indicates the red color intensity. The redness of meat is an essential quality parameter and might be affected by the processing of the meat; heat (destroys myoglobin), chilling (retains redness), and modified atmosphere packaging (preserves redness) (Orkusz et al., 2011). Poultry meat color is also determined by lightness (L*) and yellow/blue coordinates (b*). Lighter meat correlates to higher water holding capacity, lower pH, and higher texture, therefore; it is generally acknowledged that the L* value is a good indicator for PSE. (pale, soft, and exudative) condition in broilers (Barbut, 1997). Measuring b* also give further information about whether a PSE condition is to be expected. Other conditions that may cause color changes include deep pectoralis myopathy (DPM), white striping (WS), and wooden breast (WB).

pH

The pH of meat varies by the species, genotype, and muscle type (breast and thigh). However, the pH of meat may also be affected by processing stress (before and during slaughter) because it impairs the maturation process by leaving low amounts of glycogen or a high content

of lactic acid in the muscles at the time of killing (Remington, 2017). A low glycogen content results in a decreased rate of pH decline, whereas a high lactic acid content results in a very rapid pH decline. The pH of a live bird is approximately 7, and post-mortem, the pH of poultry declines to approximately 6. Meat with lower pH can be pale with lower water holding capacity. Barbut (1997) reported that chicken breasts with lower pH lose more weight due to drip loss. The pH can also be an indicator to assess the quality of the meat maturation process. If it was run "normally," the ultimate pH of the meat should be around 5.8.

Rancidity

Another factor affecting the quality of chicken breast is lipid oxidation. Lipids are essential components of all types of meat and are responsible for many desirable characteristics such as flavor and aroma profiles and contribute to tenderness and juiciness (Amal et al., 2018). The oxidation of lipids affects color, texture, nutritional value, taste, and aroma leading to rancidity and possible consumer rejection (Lima et al., 2013). The development of oxidative rancidity begins after the blood flow is interrupted during slaughter. Oxidative rancidity is a complex process where an unsaturated fatty acid reacts with a molecule of oxygen via a free-radical-chain, forming peroxides. The first oxidation leads to a chain reaction producing lipid degeneration and oxidative rancidity products (Min et al., 2005). Nature and relative proportion of the oxidative rancidity products depend on the characteristic lipid composition, processing methods, storage conditions, presence of antioxidants, and the diet of the bird before slaughter (Min et al., 2005). Malondialdehyde (MDA.) is a relatively stable secondary product of the oxidative degradation of polyunsaturated fatty acids (PUFAs). It is a three-carbon dialdehyde that can exist in various forms depending on the pH and is essential for industry and scientific research since it can determine lipid peroxidation through the Thiobarbituric Acid Reactive Substance Assay (TBARS) (Lima et

al., 2013; Min et al., 2005). Several procedures such as modified atmosphere packaging (MAP) and antioxidants have been added to increase the shelf-life by reducing the oxidative rancidity in meat (Lima et al., 2013).

EDIBLE ANTIMICROBIAL COATINGS IN FOOD

History of Edible Films and Coatings

Producers have been exploring emerging technologies to preserve meat without affecting quality (Mellinas et al., 2016; Realini et al., 2016). The main factors that affect shelf-life are contamination by spoilage and pathogenic bacteria, which could cause severe health problems to consumers. Furthermore, pathogens can modify the odor, flavor, color, and textural properties of fresh food (Gyawali et al., 2014). Previously, techniques such as heat treatment, salting or acidification, have been applied in the food industry to reduce spoilage (Lucera et al., 2012).

Edible coatings and films serve as an alternative emerging technology to increase the shelf life of food products. Edible films and coatings have unique properties such as biodegradability, consumption feasibility, free of chemical substances, and can serve as vehicles for antimicrobials. Edible antimicrobial coating has been shown to increase the shelf life of meat by reducing the proliferation of spoilage microorganisms and increasing the lag-phase or inactivating target microorganisms.

The application of coatings and films as edible components in food is not new; as early as the 12th century, citrus fruits were maintained by placing them in molten wax boxes before sending them to the Chinese Emperor (Hardenburg et al., 1967). In the 16th century, fat coatings were used to prevent shrinkage in meats (Biquet et al., 1988). In the 19th century, gelatin was introduced as a coating film to preserve various meat products (Biquet et al., 1988). Later in the 19th century, coatings became more common, and some of the popular coatings included lipids (waxes), proteins

(collagen), polysaccharides (Carrageenan) and carbohydrates (sucrose) (Labuza et al., 1981; Oskar et al., 1936; Pearce et al., 1949; Debeaufort et al., 1998). The first incorporation of an antifungal and antibiotic compound occurred in 1959 in a carrageenan film to reduce bacteria and fungi growth (Meyer et al., 1959). In 1990 sorbic acid and potassium sorbate were incorporated into edible films as antimicrobial agents (Vojdani et al., 1990). In the same year, chitosan with organic acids was first introduced as an antimicrobial film (Gennadios et al., 1994).

Edible Films and Coating

Proteins, Lipids, polysaccharides, and carbohydrates individually or in the composite form are the most used edible films and coatings. These coatings can be directly extracted from biomass and efficiently processed to form a film used as a coating. Coatings control gas exchange, moisture permeation, or oxidation while reducing or preventing the growth of microorganisms. Additionally, biopolymer films form the basis of active packaging systems by hosting additives and nutrients to be released at a controlled rate to food (Campos et al., 2011). Essential oils derived from plants, organic acids, nisin or natamycin from microbial sources, enzymes obtained from animal sources, proteins, and polymers have been proposed as active agents against microorganisms (Elsabee et al., 2013).

Protein Coatings and Films

Protein-based edible films and coatings are the most widely used material among biodegradable coatings. Proteins form excellent coatings due to the distribution of polar and non-polar charges. The differences in charges create various chemical abilities such as long lifetime, cohesive matrix, emulsifying, resistance to water penetration, radical scavenging, and antihypertensive features (Aimutis, 2004). Fish fillets coated with egg albumin and soy protein concentrate had significantly higher moisture retention (Sathivel, 2005), while Pena-Ramos

reported that a whey and soy protein coating delayed lipid oxidation in pork patties. Gelatin coating reduced the color deterioration of coated beef and pork (Antoniewski, 2007). Another distinct advantage of protein-based films is their mechanical stability. Many studies have proven that proteins preserve the integrity of edible films as compared to polysaccharides and lipids (Zinoviadou et al., 2010; Han et al., 2007).

Lipid Coatings and Films

The primary purpose of developing hydrophobic lipid coatings is to limit the moisture migration in foods. However, they are other benefits such as gas permeation and controlling flavor release. In the food industry, lipids are used to preserve color and reduce surface adhesiveness. However, this type of coatings has problems such as lack of homogeneity, cracking, greasy surface, waxy taste, and rancid odor (Ojah et al. 2010). McNally conducted a study where dipped whole chickens were dipped into corn oil, molten wax, mineral oil, and lard before freezing. The results show that the coating, especially oil and wax, significantly reduces moisture loss. Lipids are often combined with proteins or hydrocolloids to increase mechanical strength (Garcia et al., 2000). Composite films are formed by laminating two or more edible films (Kerry et al., 2005; Perez-Gago et al., 2000). Ben and Kurth, (1995) developed a casein lipid composite film, which improved the juiciness and appearance of meat. Ojagh et al., (2010) incorporated cinnamon oil into a chitosan film that reduced food's oxidative rancidity. Other lipids such as monoglycerides and diglycerides have also significantly reduced of oxidative rancidity in meat and salmon fillets.

Polysaccharide Coatings and Films

Polysaccharide films have three main advantages: (i) they possess oxygen barrier properties, (ii) act as sacrificing agents, (iii) and provide appealing appearances (Valdes et al., 2017). Wu et al. (2000) showed that using a starch alginate film significantly ($p < 0.05$) reduces

moisture loss and lipid oxidation in ground beef patties during a six-day storage period. Polysaccharide films minimize water loss due to their hygroscopic nature, meaning that they attract and hold water. Despite being hygroscopic polysaccharides, films are also permeable to water (Valdes et al., 2017). Polysaccharides are also known as "sacrificing agents." An example of a "sacrificing agent" is a Carrageenan/Alginate solution that forms a gel that intentionally absorbs water and protects against excessive water loss (Shaw et al., 1980; William et al., 1978). Additionally, polysaccharide coatings prevent gas penetrations, especially oxygen. The presence of oxygen is detrimental because it can lead to oxidative rancidity and the growth of spoilage bacteria. Khan et al. (2009) developed a pectin coating that increased the moisture content and reduced TBARS (measurement of rancidity) and microbial population on irradiated buffalo meat patties. Finally, polysaccharides films are typically colorless or slightly yellow. The slight yellowness is preferable compared to other films that cause off-colors due to Maillard reactions (Trezza et al., 2000).

Carrageenan/Chitosan Coatings

Polysaccharides such as Chitosan and Carrageenan are competitive candidates for forming nano-layered coatings due to their opposite electrostatic properties, bioactive and non-toxic properties. Chitosan is a natural polysaccharide obtained by deacetylation of chitin, which is the major constituent of the exoskeleton of crustaceous animals and has been extensively used to produce biodegradable films (Shi et al., 2009). Chitosan is an excellent film component due to its good oxygen barrier properties and due to its intrinsic antimicrobial activity (Begin et al., 1999). Carrageenan is a sulfated polysaccharide extracted from certain red seaweeds. It is extensively used in the food industry as a gelling, emulsifying, and stabilizing agent and has been reported as having excellent film-forming properties. A Carrageenan/Chitosan coating combination is

believed to form a strong nanolayer coating mainly due to the electrostatic interactions existing between the two polyelectrolytes. A previous study conducted by Olaimat et al. (2014) concluded that a Carrageenan/Chitosan coating with AITC reduces the Salmonella and Campylobacter populations on the chicken breast by 2.3 and 1.75 log CFU/g 4 °C by day 21(Olaimat et al., 2014). A layer containing Carrageenan/Chitosan and Niacin effectively killed over 90% and 99% of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) (Webber et al., 2021).

Application of Antimicrobial Edible Coatings

Spraying

Edible coatings are applied by spraying, dipping, and spreading. Spraying is of interest to food processors because of the potential cost reduction and the high quality of the final product compared to other conventional techniques (Andrade et al., 2012). Spraying techniques offer a uniform coating, thickness control, and the possibility of multilayer application (Martin-Belloso et al., 2009; Ustunol et al., 2009). Spraying systems also do not contaminate the coating. The droplet size while using sprayers can be as small as 20 µm, increasing the surface area of the coating and forming a coating with an even surface. On the other hand, coatings with more than one layer may require multiple sprayers and drying steps.

Spreading

Spreading, also known as brushing, applies coating onto a surface by using a blade attached to the lower part of a spreading device. The use of an edge allows for control of the film thickness. This method can be applied to the production of polysaccharides and protein-based films (Mendez-Villas, 2013). Spreading can be affected the surface texture, environmental conditions, and liquid properties (Khan et al., 2009).

Dipping

Dipping techniques form thick membranous films over the product surface by directly dipping the product into the aqueous coating formulation and further air-drying. The process happens in three stages immersion, deposition (draining of liquid), evaporation (solvent excess evaporates). The ideal amount of coating solution cannot be easily controlled with the dipping technique. Further processing steps such as drying are needed, requiring extra time, and hindering the industrial application.

Dipping can also be applied using the layer-by-layer technique. This technique has proven to be an effective technique due to the possibility of controlling the antimicrobial release and incorporating a wide range of biological functions (Silva-Buzanello, 2019). The layer-by-layer technique alternates polyelectrolytes with different functional moieties with functional molecules (Giese, 2003). Studies have shown that alternating K-carrageenan/Chitosan layer to up to six layers creates a multilayer system that can control the release of the active compounds (SSD, 2021). Several layers need to be added before the active components are added. Adding the antimicrobial as the last layer is ideal because several K-carrageenan/Chitosan coating layers need to be added to prevent high interpenetration between layers. This phenomenon may lead to uneven deposition in the first layer of the nanostructure and thus less charged chain segments for adhesion of antimicrobial (TTC, 2010). Studies have also shown that the antimicrobials' ability to diffuse across the membrane increases when there is a low interpolation between layers.

Antimicrobial Coatings

In meat products, the highest level of contamination occurs on the surface of the product. Incorporating antibacterial materials in edible films has been considered a giant leap in reducing pathogenic bacteria and spoilage (Du et al., 2011). The antimicrobial agents used in food vary and

include proteins, lipids, organic acids, or combinations. The following sections discuss some of the antimicrobials that have been applied to meats.

Organic Acids

Organic acids are Generally Regarded as Safe (GRAS) and either exist naturally in fruits and vegetables or are synthesized by microorganisms via fermentation (Shin et al., 2017). Organic acids and their salts are used as antimicrobial agents due to their well-known effectiveness and low cost. Organic acids have some advantages, such as low cost, approval by the current European legislation, and simple manipulation without changing the organoleptic characteristics of the poultry product (Shin et al., 2017). Organic acids in the undissociated form can penetrate the cell membrane and dissociate inside the cell. The dissociation causes an increase in the proton concentration, thereby decreasing the external pH and impacting the cell. The standard organic acids integrated into edible coatings include lactate, acetate, propionate, p-aminobenzoic acid, and malic acid. In a study, whey protein films incorporated with grape seeds, malic acid, and nisin on turkey reduced *L. monocytogenens* and *S.* Typhimurium by 2.3 and 5 log CFU/g after 28 days at 4°C (Bassole et al., 2012).

Bacteriocins

Bacteriocins are known as ribosomal synthesized, proteinaceous toxins produced by bacteria. Bacteriocins destroy other closely related microorganisms through numerous mechanisms such as plasmids and conjugative transposons (Inglis et al., 2013; Micenkova et al., 2014; Yamashita et al., 2011). There are many different types of bacteriocins, varying in their microbial action (Ahmad et al., 2017). Bacteriocins are not widely used in the poultry industry. However, recent research done by Kim et al. (2019) showed that a film containing niacin and lacticin could slow down the growth rate of coliforms and the total aerobic bacteria in oysters and

ground beef. Nisin can penetrate the cytoplasmic membrane of bacteria, causing the leakage of cytoplasmic contents and dissipation of the membrane potential (Gharcallaoui et al., 2016).

Proteins

Proteins such as amino acid-structured antimicrobials (enzymes), nutrient binding proteins, and smaller antimicrobials peptides can act as antimicrobials by disrupting the structure of the microbial cell membrane. An example of an antimicrobial protein is lysozyme which disrupts the cell wall via hydrolysis. Research has shown that whey protein films combined with lysozyme effectively reduce the bacteria population in salmon slices (Balciunas et al., 2013; Gomez-Estaca et al., 2010).

Herbs and Spices

The antimicrobial activity of different spices and herbs has been known since ancient times, and they have been traditionally added to food as a seasoning additive due to their aromatic properties (Valdes et al., 2015). In the last 20 years, essential oils (Eos) antiseptic properties have been evaluated by many researchers. Eos are defined as the product obtained from raw plants and have advantages such as: anti-cancer, anti-inflammatory, diabetic, antiulcer genic, antidepressant, and antianxiety (Anderson et al., 2017; Ribero-Santos et al., 2017). Eos are concentrated hydrophobic liquids with volatile aroma compounds such as terpenes, terpenoids, and aliphatic chemicals. These phenolic groups are responsible for damage to the cell wall, interaction with and disruption of the cytoplasmic membrane, damage of membrane proteins, leakage of cellular components, coagulation of cytoplasm, and depletion of the proton motive force (Ramos et al., 2017). All these effects produce death to microorganisms by modifying the structural composition of the bacteria cell walls (Cao et al., 2009). Essential oils may change the product's organoleptic properties and can be difficult to integrate due to their hydrophobic nature. In research, oregano

essential oils have been shown to decrease *L. monocytogenes* population by 2.4 log CFU/g after 28 days at 4°C (Balciunas et al., 2013). Also, grapefruit seed extract added into the antimicrobial films has been found to prevent *E. coli* and *L. monocytogenes* growth in pork (Yamashita et al., 2011). Additionally, Matiacevich et al. (2015) studied the effect of alginate-based edible coatings with propionic acid and thyme essential oil on the microbiological growth in chicken breast fillets stored under refrigerated conditions, demonstrating the inhibition of *Salmonella* after seven days.

Allyl Isothiocyanate

Allyl isothiocyanate (AITC) is a volatile and aliphatic sulfur-containing compound naturally occurring in plants from the family of Cruciferae. AITC is the primary flavor component in many foods such as wasabi, horseradish, and mustard. It also exhibits potent antimicrobial activity against Gram-Positive, Gram-Negative bacteria and fungi (Winther, M. et al., 2006) AITC is formed when glucosinolates are cleaved/hydrolyzed. AITC is considered a GRAS substance in the United States. In Europe, the use of AITC as a food additive is still under review. Few studies have reported on the application of AITC in food packaging mainly due to its strong odor. However, studies have shown that gaseous isothiocyanate causes metabolite leakage, measurable increases in 3-galacosidase activity, and reduction of viable bacteria by a 1,000-fold at 25 µg/mL (Lin et al., 2000).

Additionally, nitriles have been shown to act as respiratory inhibitors in bacteria leading to cell death (Matiacevich et al., 2015). AITC impregnated filter paper disks reduced the growth of *Escherichia coli* O157:H7 and extended the shelf life of refrigerated ground beef (Nadarajah et al., 2005). AITC also reduced *Listeria monocytogenes* in freshly cut onions (Nadarajah et al., 2005). A study conducted by Olaimat et al. (2014) concluded that a Carrageenan/Chitosan coating with

AITC reduces the *Salmonella* and *Campylobacter* populations on the chicken breast by 2.3 and 1.75 CFU/g by 21 days at 4°C (Olaimat et al., 2014).

REFERENCES

Ahmad, V., Khan, M. S., Jamal, Q. M. S., Alzohairy, M. A., Al-Karaawi, M. A., & Siddiqui, M. U. (2017). Antimicrobial potential of bacteriocins in therapy, agriculture and food preservation. *International Journal of Antimicrobial Agents, 49,* 1-11. https://doi.org/10.1016/j.ijantimicag.2016.08.016

Aho, P. (2021). *US Boneless, Skinless, Chicken Breast Industry Continues to Grow*. FeedStrategy. https://www.feedstrategy.com/uncategorized/us-boneless-skinless-chicken-breast-industry-continues-to-grow/

Aimutis, W. R. (2004). Bioactive properties of milk proteins with particular focus on anticariogenesis. *The Journal of Nutrition, 134*, 989-995. https://doi.org/10.1093/jn/134.4.989s

Amaral, A. B., da Silva, M. V., & Lannes, S. C. (2018). Lipid oxidation in meat: mechanisms and protective factors. *Food Science and Technology, 38,* 1-15. https://doi.org/10.1590/fst.32518

Anderson, R. A., Zhan, Z., Luo, R., Guo, X., Guo, Q., Zhou, J., Kong, J., Davis, P. A., & Stoecker, B. J. (2017). Cinnamon extract lowers glucose, insulin and cholesteron in people with elevated serum glucose. *Journal of Traditional and Complementary Medicine, 6,* 332-336. https://dx.doi.org/10.1016%2Fj.jtcme.2015.03.005

Andrade, R. D., Skurtys, O., & Osario, F. A. (2012). Atomizing spray systems for application of edible coatings. *Comprehensive Reviews in Food Science and Food Safety, 11,* 323-337.

Antoniewski, M. N., Barringer, S. A., Knipe, L., & Zerby, H. N. (2007). Effect of a gelatin coating on the shelf life of fresh meat. *Journal of Food Science, 76*, 382-387. http://dx.doi.org/10.1111/j.1750-3841.2007.00430.x

Balciunas, E. M., Castillo, F. A., Todorov, S., de Melo Franco, B., Converti, A., & de Souza Oliveira, R. P. (2013). Novel biotechnological applications of bacteriocins: a review. *Food Control*, *32*, 134-142. http://dx.doi.org/10.1016/j.foodcont.2012.11.025

Barbut, S. (1997). Problem of pale soft exudative meat in broiler chickens. *British Poultry Science, 38*, 355-358. https://doi.org/10.1080/00071669708418002

Barnes, E. M. (1976). Microbiological problems of poultry at refrigerator temperatures-a review. *Journal of the Science of Food and Agriculture*, *27*, 777-782. https://doi.org/10.1002/jsfa.2740270813

Barnett R. (2016). Typhoid Fever. *Lancet, 388*, 2467. https://doi.org/10.1016/s0140-6736(16)32178-x

Bassole, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules, 17*, 3989-4006. https://doi.org/10.3390/molecules17043989

BCcampus. (2018). *Poultry Cuts*. BCcampus. https://opentextbc.ca/meatcutting/chapter/poultry-cuts/

Begin, A., & Van Calsteren, M. R. (1999). Antimicrobial films produced from chitosan. *International Journal of Biological Macromolecules*, *26*, 63-67. https://doi.org/10.1016/S0141-8130(99)00064-1

Bennett, P. (2008). Compliance guidelines for controlling Salmonella and Campylobacter. *Poultry*, *2*, 234-240.

Berkhout N. (2020). Popularity of Poultry Continues Globally. *Poultry World*.

Berrang, M. E., Buhr, R. J., Cason, J. A., & Dickens, J. A. (2001). Broiler carcass contamination with *Campylobacter* from feces during defeathering. *Journal of Food Protection, 64*, 2063-2066. *https://doi.org/10.4315/0362-028x-64.12.2063*

Bilgili, S.F. (1998). "Zero Tolerance" begins at the farm. *Broiler Industry,* 61(11). 30.

Biquet, B., & Labuza, T. P. (1988). Evaluation of the moisture permeability characteristics of chocolate films as an edible moisture barrier. *Journal of Food Science, 53*, 989-998. https://doi.org/10.1111/j.1365-2621.1988.tb13516.x

Britannica. (2020). *Livestock*. Britannica. https://www.britannica.com/animal/livestock

Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., & Swaminathan, B. (2000). *Salmonella* nomenclature. *Journal of Clinical Microbiology, 38*, 2465-2467. https://doi.org/10.1128/jcm.38.7.2465-2467.2000

Buzby, J. C., Wells, H. F., Axtman, B. & Mickey, J. (2009). *Supermarket loss estimates for fresh fruit, vegetables, meat, poultry, and sea food and their use in the ERS loss-adjusted food availability data*. United States Department of Agriculture Economic Research Service. https://www.ers.usda.gov/publications/pub-details/?pubid=44309

Campos, C. A., Gerschenson, L. N., & Flores, S. K. (2011). Development of edible films and coatings with antimicrobial activity. *Food and Bioprocess Technology, 4*, 849-875. https://doi.org/10.1007/s11947-010-0434-1

Center of Disease Control, (2019). *Estimates of Food Illness in the United States*. Center of Disease Control. https://www.cdc.gov/foodborneburden/index.

Center of Disease Control. (2019). *Information for Health Professionals.* Center of Disease Control. https://www.cdc.gov/flu/professionals/index.htm

Chantarapanont, W., Berrang, M., & Frank, J. F. (2003). Direct microscopic observation and viability determination of *Campylobacter jejuni* on chicken skin. *Journal of Food Protection, 66*, 2222-2230. https://doi.org/10.4315/0362-028x-66.12.2222

Clark, A. G., & Bueschkens, D. H. (1985). Laboratory infection of chicken eggs with *Campylobacter jejuni* by using temperature or pressure differentials. *Applied Environmental Microbiology, 49*, 1467-1471. https://doi.org/10.1128/aem.49.6.1467-1471.1985

Cunningham, D. L. (1997). Contract Broiler grower returns, a long-term assessment. *Journal of Applied Poultry Research, 6*, 267-273. https://doi.org/10.1093/japr/6.3.267

Cunningham, D. L., & Fairchild, B. D., (2009-2010). Broiler production system in Georgia costs and returns analysis. *The University of Georgia Cooperative Extension CAES.*

Crushell, E., Harty, S., Sharif, F., & Bourke, B. (2004). Enteric *Campylobacter:* purging its secrets. *Pediatric Research, 55*, 3-12. https://doi.org/10.1203/01.pdr.0000099794.06260.71

Crump, J. A., Sjolund-Karlsoon, M., Gordon, M. A., & Parry, C. M. (2015). Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive *Salmonella* Infections. *Clinical Microbiology Review, 28*, 901-937. https://dx.doi.org/10.1128%2FCMR.00002-15

Coburn, B., Grassl, G. A., & Finlay, B. B. (2007). *Salmonella,* the host and disease a brief review. *Immunology Cellular Biology, 85*, 112-118. https://doi.org/10.1038/sj.icb.7100007

Dasti, J. I., Tareen, A. M., Lugert. R., Zautner, A. E., & Gro, U. (2010). *Campylobacter jejuni:* A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology, 300*, 205-211. https://doi.org/10.1016/j.ijmm.2009.07.002

Davis, C. G., Zahnise, F., & Gale, W. (2019). *Assessing the Growth of U.S Broiler and Poultry Meat Exports.* United States Department of Agriculture.

Debeaufort, F., Quezada-Gallo, J. A., & Voilley, A. (1998). Edible films and coatings: tomorrow's packaging: a review. *Critical Review in Food Science, 38*, 299-313. https://doi.org/10.1080/10408699891274219

Diaz-Sanchez, S., Hawkins, R., Okimoto, R., Layton, A., Saxton, A., Blakeley-Ruiz, A. & Hanning, I. (2010). *Correlation of Microbiome Populations with Performance Measurements and Gender in Broiler Breeders,* Boston, MA: Congress of Gastrointestinal Function.

Du, W. X., Avena-Bustillos, R. J., Hua, S. S. T., & McHugh, T.H. (2011). Antimicrobial volatile essential oils in edible films for food bacteriocin. *Antimicrobial agents and chemotherapy, 55*, 4352-4360

Venable, G. (2021). *Economic Impact of the Poultry Industry*. Poultry Perspectives. https://www.provisioneronline.com

EFSA. (2011). Scientific opinion on *Campylobacter* in broiler meat production: Control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal, 9*, 2105-2246. https://doi.org/10.2903/j.efsa.2011.2105

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings. *Material Science and Engineering, 33*, 1819-1841. https://doi.org/10.1016/j.msec.2013.01.010

Fabrega, A., & Vila, J. (2013). *Salmonella* entérica serovar Typhimurium skills to succeed in the host: virulence and regulation. *Clinical Microbiology Review, 26*, 308-341. https://doi.org/10.1128/cmr.00066-12

Feasey, N.A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal *Salmonella* disease: An emerging and neglected tropical disease in Africa. *Lancet, 379,* 2489-2499. https://dx.doi.org/10.1016%2FS0140-6736(11)61752-2

Fernandez, H., Vera, F., Villanueva, M. P., & Garcia, A. (2008). Occurrence of *Campylobacter* species in healthy well-nourished and malnourished children. *Brazilian Journal of Microbiology, 39,* 56-58. https://doi.org/10.1590/s1517-838220080001000013

Fleet, G. H., & Praphailong, W. (2001). Spoilage of processed foods: causes and diagnosis. *Food microbiology,* 382-397.

Foster, J. W., & Hall, H. K. (1991). Inducible pH homoestasis and acid tolerance response of *Salmonella* Typhimurium. *Journal of Bacteriology*, *173*, 5129-5135. https://dx.doi.org/10.1128%2Fjb.173.16.5129-5135.1991

Fremery, D., & Pool, M. F. (1960). Biochemistry of chicken muscle as related to rigor mortis and tenderization, *Journal of Food Safety*, *25*, 73. https://doi.org/10.1111/j.1365-2621.1960.tb17938.x

Friedman, C. R. Neimann, J., Wegener, C. W., & Tauxe, R. V. (2000). Epidemiology of *Campylobacter* jejuni infections in the United States and other industrialized nations. *Campylobacter*.

Gadang, V. P., Hettiarachchy, N. S., Johnson, M. G., & Owens, C. (2008). Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *Journal of food science, 73,* 389-394. https://doi.org/10.1111/j.1750-3841.2008.00899.x

Garcia-del Portillo, F., & Finlay, B. B. (1995). Targeting of *Salmonella* Typhimurium to vesicles containing lysosomal membrane glycoproteins bypasses compartments with mannose 6-phosphate receptors. *Journal of Cellular Biology, 129*, 81-97. https://doi.org/10.1083/jcb.129.1.81

Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2006). Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journals of Food Science, 65*, 941-944. https://doi.org/10.1111/j.1365-2621.2000.tb09397.x

Gashorn, M. A., & Serini, C. (2006). Quality of chicken meat from conventional and organic production. *Department of Poultry Science University of Hohenheim.*

Gast, R. K., Guraya. R., Guard-Bouldin, J., Holt, P. S., & Moore, R. W. (2007). Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs laid by hens infected with *Salmonella* Enteritidis of *Salmonella* Heidelberg. *Avian Diseases, 51*, 40-44. https://doi.org/10.1637/0005-2086(2007)051[0040:cosrot]2.0.co;2

Gennadios, A, & McHugh, C. T. (1994). Edible coatings and films based on proteins. *Edible Coatings and Films to Improve Food Quality.*

Georgia Poultry Industry. (2021). *Georgia's Poultry Industry and its Impact on the Local Economy and Global Trade.* United States Congress.

Gharcallaoui, A., Oulahal, N., Joly, C., & Degraeve, P. (2016). Nisin as a food preservative. Critical *Review in Food Science and Nutrition, 56,* 1262-1274. https://doi.org/10.1080/10408398.2013.763765

Giese, J. (2003). *Color measurements in foods*. Food Technology Magazine. *https://www.ift.org/news-and-publications/food-technology-magazine/issues/2003/december/columns/laboratory*

Golz, G., Rosner, B., Hofreuter, D., Josenhans, C., Kreienbrock, L., Lowenstein, A., Schielke, A., Stark, K., Suerbaum, S., & Wieler, L. H. (2014). Relevance of *Campylobacter* to public health- The need for one health approach. *International Journal of Medical Microbiology, 304*, 817-823. https://doi.org/10.1016/j.ijmm.2014.08.015

Gomez-Estaca, J., de Lacey, A. L., Lopez-Caballero, M. E., Gomez-Guillen, M. C., & Montero, P. (2010). Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food microbiology, 27*, 889-896. https://doi.org/10.1016/j.fm.2010.05.012

Gulliespie, I. A., O'Brien, S. J., Adak, G. K., Tam, C. C., Frost, J. A., & Bolton, F. J. (2003). Point source outbreak of *Campylobacter jejuni* infections—are they more common than we think and what might cause them? *Epidemiology and infections, 130*, 367-375.

Gyawali, R., & Ibrahim, S. A., (2014). Natural products as antimicrobial agents. *Food Control*, 46, 412-429. https://doi.org/10.1016/j.foodcont.2014.05.047

Haddad, N., Burns, C. M., Bolla, J. M., Prevost, H., Federighi, M., Drider, D., & Capppelier, J. M. (2009). Long-term survival of *Campylobacter jejuni* at low temperatures is dependent on polynucleotide phosphorylase activity. *Applied and Environmental Microbiology, 75*, 7310-7318. https://doi.org/10.1128/aem.01366-09

Han, J. H., & Krochta, J. M. (2007). Physical properties of whey protein coatings solution and films containing antioxidants. *Journal of Food Science, 75*, 308-314. https://doi.org/10.1111/j.1750-3841.2007.00358.x

Hardenburg, R. E. (1967) Wax and related coatings for horticultural products. *U.S Department of Agriculture Research Service.* 1-15.

Hinton, A., Carson J. A., & Ingram, K. D. (2004). Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. *International Journal of Food Microbiology,* 91, 155-165.

Humphrey, T., O'Brien, S., & Madsen, M. (2007). Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*, *117*, 237-257. https://doi.org/10.1016/j.ijfoodmicro.2007.01.006

Holl, L., Behr, J., & Vogel, R. F. (2016). Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat. *Food Microbiology*, *60*, 85-91. https://doi.org/10.1016/j.fm.2016.07.003

Inglis, R. F., Bayramoglu, B., Gillor, O., & Ackermann, M. (2013). The role of bacteriocins as selfish genetic elements. *Biology Letters, 9*, 121-173. https://doi.org/10.1098/rsbl.2012.1173

Jay, J. M. (2005). Lactic Acid Bacteria. *Microbiologia de Alimentos.*

Joshua, G. W. P., Gunthrie-Irons, C., Karlyshev, A. V., & Wren, B. W. (2006). Biofilm formation in *Campylobacter jejuni. Microbiology,* 152, 387-396. https://doi.org/10.1099/mic.0.28358-0

Kaakoush, N. O., Castano-Rodriguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Review*, *28,* 687-720. https://doi.org/10.1128/cmr.00006-15

Kerry, J. F., & Liu, L. (2005). Selection of optimum extrusion technology parameters in the manufacture of edible/biodegradable packaging films derived from food-based polymers. *International Journal of Food, Agriculture, and Environment, 3,* 51-58.

Kester, J., & Fennema, O. (1986). Edible films and coatings: a review. *Food Technology, 40,* 47-59.

Khan, M. I., & Nasef, M. M. (2009). Spreading behavior of silicone oil and glycerol drops on coated papers. *Leonardo Journal of Science, 14*, 18-30.

Kim, K. Y., Frank, J. F., & Craven, S. E. (1996). Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Letter in Applied Microbiology, 22*, 280-282. https://doi.org/10.1111/j.1472-765x.1996.tb01161.x

Labuza, T., & Contreras-Medellin, R. (1981). Prediction of moisture protection requirements for foods. *Cereal Foods World.*

Lawrie, R. A., & Ledward, D. A. (2006). Lactic Acid Bacteria. *Lawrie's meat science, 7,* 350-400.

Lebret, B., Prevolnik, P., & Candek-Potokar, M. (2015). A handbook of reference methods for meat quality. *European Cooperation in Science and Technology.*

Lee, M. D., & Newell, D. G. (2006). *Campylobacter* in poultry: filling an ecological niche. *Avian Diseases, 50,* 1-9. https://doi.org/10.1637/7474-111605r.1

Lillard, H. S. (1990). The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. *Journal of Food Protection, 53,* 202-207. https://doi.org/10.4315/0362-028x-53.3.202

Lima, D. M., Rangel, A., Urbano, S., Mitzi, G., & Moreno, G.M. (2013). Lipid oxidation in beef. *Acta Veterinaria Brasilica, 7*, 14-28.

Lin, C. M., Preston, J. F., & Wei, C. I. (2000). Antimicrobial mechanism of allyl isothiocyanate. *Journal of food protection, 63,* 727-734. https://doi.org/10.4315/0362-028x-63.6.727

Lucera, A., Costa, C., Conte, A., & Del Nobile, M. A. (2012). Food applications of natural antimicrobial compounds. *Frontiers in Microbiology, 3,* 287. https://doi.org/10.3389/fmicb.2012.00287

Magajna, B. A., & Sharaft, H. (2015). *Campylobacter jejuni* biofilm cells become viable but non-culturable (VBNC) in low nutrient conditions at 4°C more quickly than their planktonic counter parts. *Food Control,* 50, 45-50. http://dx.doi.org/10.1016%2Fj.foodcont.2014.08.022

Marder, E. P., Cieslak, P. R., Cronquist, A. B., Dunn, J., Lathrop, S., & Rabasky-Her, T. (2017) Incidence and trends of infections with pathogens transmitted commonly though food and the effects of increasing use of culture-independent diagnostic tests on surveillance. *Foodborne Diseases Active Surveillance*. https://doi.org/10.15585/mmwr.mm6615a1

Moore, D., Robson, D. G., & Trinci, P. J. (2011). 21st Century guidebook to fungi. *Cambridge University Press.*

Martin-Belloso, O., Rojas-Grau, M. A., & Soliva-Fortuny, R. (2009). Delivery of flavor and active ingredients using edible films and coatings. *Edible Films and Coatings for Food Applications,* 295-314. https://doi.org/10.1007/978-0-387-92824-1_10

Matiacevich, S., Acevedo, N., & Lopez, D. (2015) Characterization of edible active coating based on alginate-thyme oil-propionic acid for preservation of fresh chicken breast fillets. *Journal Food Processing,* 39, 2792-2801. https://doi.org/10.1111/jfpp.12530

Mellinas, C., Valdes, A., Ramos, M., Burgos, N., Garrigos, M. C., & Jimenez, A. (2015). Active edible films: Current state and future trends. *Journal of Applied Polymer Science, 133,* 180-200. https://doi.org/10.1002/app.42631

Micenkova, L., Staudova, B., Bosak, J., Mikalova, L., Littenerova, S., Vrba, M., Sevcikova, A., Woznicova, V., & Smajs, D. (2014). Bacteriocin encoding genes and ExPEC

virulence determinants are associated in human fecal Escherichia coli strains. *BMC microbiology, 14*, 109. https://doi.org/10.1186/1471-2180-14-109

Min, B., & Ahn, U. (2005). Mechanisms of lipid peroxidation in meat and meat products-a review. *Food Science and Biotechnology,* 14(1), 152-163.

Monteville, M. R., Yoon, J. E., & Konkel, M. E. (2003). Maximal adherence and invasion of INT 407 cells by *Campylobacter jejuni* requires the CadF outer-membrane protein and microfilament reorganization. *Microbiology, 149*, 153-165. https://doi.org/10.1099/mic.0.25820-

Moran, E.T. (1999). Live production factors influencing yield and quantity of poultry meat, *Poultry Science Symposium Series,* Volume 25, Wallingford, Oxon, U.K.

Nachamkin, I., Szymanski, C. M., & Blaser, M. J. (2008). *Campylobacter (*3rd ed.). Washington, DC: ASM Press.

Nadarajah, D., Han, J. H., & Holley, R. A. (2005). Inactivation of Escherichia coli O157:H7 in packaged ground beef by allyl isothiocyanate. *International Journal of Food Microbiology, 99*, 269-279. https://doi.org/10.1016/j.ijfoodmicro.2004.08.019

Nde, C. W., McEvoy, J. M., Sherwood, J. S., & Logue, C. M. (2007). Cross contamination of turkey carcasses by *Salmonella* species during defeathering. *Poultry Science, 86,* 162-167. https://doi.org/10.1093/ps/86.1.162

Newell, D. G., Elvers, K. T., Dopfer, D., Hansson, I., Jones, P., James, S. & Allen, V. M. (2011). Biosecurity-Based interventions and strategies to reduce *Campylobacter spp.* on poultry farms. *Applied and Environmental Microbiology, 77,* 8605-8614. https://doi.org/10.1128/aem.01090-10

Nieminen, T. T., Koskinen, K., Laine, P., Hultman, J., Sade, E., Paulin, L., Paloranta, A., Johansson, P., & Auvinen, P. (2012). Comparison of microbial communities in marinated and

unmarinated broiler meat by metagenomics. *Int. J. Food Microbiol, 157,* 142-149. https://doi.org/10.1016/j.ijfoodmicro.2012.04.016

Nilsson, A., Johansson, C., Skarp, A., Kaden, R., Engstrand, L., & Rautelin, H. (2017). Genomic and phenotypic characteristics of Swedish *C. jejuni* water isolated. *PLOS ONE. 12.* https://doi.org/10.1371/journal.pone.0189222

Northcutt, J. K., Savage, S. I., & Vest, L.R. (1997). Relationship between feed withdrawal and viscera condition of broilers, *Poultry Science,* 76, 410-414. https://doi.org/10.1093/ps/76.2.410

Oakley, B. B., Lillehoj, H. S., Kogurt, M. H., Kim, W. K., Maurer, J. J., & Pedroso, A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters*, 36, 100-112. https://doi.org/10.1111/1574-6968.12608

Oh, E., McMullen, L. M., Chui, L., & Jeone, B. (2017). Differential survival of hyper-aerotolerant *Camyplybacter jejuni* under different gas conditions. *Frontiers in Microbiology, 8, 954.* https://doi.org/10.3389/fmicb.2017.00954

Ojagh, S. M., Rezai, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry, 120*, 193-198. https://doi.org/10.1016/j.foodchem.2009.10.006

Okoro, C. K., Kingsley, R. A., Connor, T. R., Harris, S. R., Parry, C. M., & Al-Mashhadani, M. N. (2012). Intracontinental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Sahara Africa. *Nature Genetics*, 44, 1215-1221. https://doi.org/10.1038/ng.2423

Olaimat, A. N., & Holley, R. A. (2015). Control of *Salmonella* on fresh chicken breast by k-carrageenan/chitosan-based coatings containing allyl isothiocynate or deodorized oriental

mustard extract plus EDTA. *Food Microbiology, 48*, 83-88. https://doi.org/10.1016/j.fm.2014.11.019

Orkusz, A., Woloszyn, J., & Okruszek, A. (2011). Characteristics of the thigh muscles color from turkey packaged under modified atmosphere in different film types. *Arch. Geflugelk,* 75, 196-203.

Oskar, W. B. (1936). Methods for working up fibrous material from hides.

Owens, C. M., Owens, C. M., & Sams, A. R. (2009). Introduction to poultry meat processing. *Poultry Meat Processing, 2*.

Pearce, J. A., & Layers, C. (1949). Frozen Storage of Poultry: V. effects of come processing factors on quality. *Canadian Journal of Research, 27*, 253-265. https://doi.org/10.1139/cjr49f-026

Pena-Ramos, E. A., & Xiong, Y. L. (2003) Whey and soy protein hydrolysates inhibit lipid oxidation in cooked pork patties. *Meat Science, 64*, 259-263. https://doi.org/10.1016/s0309-1740(02)00187-0

Perez-Gago, M. B., & Krochta, J. M. (2001). Lipid particle size effect on water vapor permeability and mechanical properties of whey protein/beeswax emulsion films. *Journal of Agriculture and Food Chemistry, 49*, 996-1002. https://doi.org/10.1021/jf000615f

Perry, C. M., Hien, T. T., Dougan, G., White, N. J., & Farrar, J. J. (2002). Typhoid Fever. *North England Journal of Medicine, 347*, 1770-1782. https://doi.org/10.1056/nejmra020201

Prescott, L. M., Harley, J. P., & Klein, D. A. (2005). Microbiology, *6*, 652-668.

Ramos, M., Jimenez, A., & Garrigos, M. C. (2017). Active nanocomposite in food contact materials. *Nanoscience in Food and Agriculture, 4,* 1-44. https://doi.org/10.1007/978-3-319-53112-0_1

Rathman, M., Barker, L. P., & Falkow, S. (1997). The unique trafficking pattern of *Salmonella* Typhimurium containing phagosomes in murine macrophages is independent of the mechanism of bacterial entry. *Infection Immunology, 65,* 1475-1485. https://doi.org/10.1128/iai.65.4.1475-1485.1997

Remignon, H., Gardaheut, M. F., Marche, G., & Ricard, F. H. (1995). Selection for rapid growth increases the number and size of muscle fivers without changing their typing in chickens. *Journal of Muscle Research and Cell Motility,* 16, 95-102. https://doi.org/10.1007/BF00122527

Remington H. (2017). Achieving sustainable production of poultry meat, Volume 1: safety, quality and sustainability. *British Poultry Science, 58,* 17. https://doi.org/10.1080/00071668.2017.1355511

Ribero-Santos, R., Andrade, M., de Melo N. R., & Sanches-Silva, A. (2017). Use of essential oils in active food packaging: Recent advances and future trends. *Trends in Food Science & Technology, 61,* 132-140. https://doi.org/10.1016/j.tifs.2016.11.021

Romero-Barrios, P., Hempen, M., Messens, W., Stella, P., & Hugas, M. (2013). Quantitative microbiological risk assessment (QMRA) of food-borne zoonoses at the European level. *Food Control, 29,* 343-349. https://doi.org/10.1016/j.foodcont.2012.05.043

Russell, S. M. (2008). The effect of an acidic, copper sulfate-based commercial sanitizer on indicator, pathogenic, and spoilage bacteria associated with broiler chicken carcasses when applied at various intervention points during poultry processing. *Poultry Science,* 87, 1435-1440. https://doi.org/10.3382/ps.2007-00339

Saez-Lara, M. J., Gomez-Llorante, C., & Gil, A. (2015). The role of probiotic lactic acid bacteria and bifidobacterial in the prevention and treatment of inflammatory bowel disease and other related diseases. *BioMedical Research International.* https://doi.org/10.1155/2015/505878

Sahin, O., Kassem, I. I., Shen, Z., Lin, J., Rajashekara, G., & Zhang, Q. (2015). *Campylobacter* in poultry: ecology and potential interventions. *Avian Diseases, 59,* 185-200. https://doi.org/10.1637/11072-032315-review

Salfinger, Y., & Tortorello, M. L. (2015). Compendium of Methods for Microbiological Examination of Foods. *American Public Health Association.*

Sathivel, S. (2006). Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of pink salmon fillets during frozen storage. *Journal of Food Science, 70,* 455-459. https://doi.org/10.1111/j.1365-2621.2005.tb11514.x

Santos, R. L., Tsolis, R. M., Baumler, A. J., & Adams, L. G. (2003). Pathogenesis of *Salmonella*-induced enteritis. *Brazilian Journal Medicine and Biology, 36,* 3-12. https://doi.org/10.1590/s0100-879x2003000100002

Shi, P., Zuo, Y., Zou, Q., Shen, J., Zhang, L., & Li, Y. (2009) Improved properties of incorporated chitosan film with ethyl cellulose microspheres for controlled release. *International Journal of Pharmaceutics, 375,* 67-74. https://doi.org/10.1016/j.ijpharm.2009.04.016

Shin, S. h., Chang, Y., Lacrox, M., & Han, J. (2017). Control of microbial growth and lipid oxidation on beef product using an apple peel-based edible coating treatment. *Food science and technology*. *84,* 182-188. https://doi.org/10.1016/j.lwt.2017.05.054

Silva-Buzzanello, R. A., Schuch, A. F., Gasparin, A. W., Torquato, A. S., Scremin, F, R., Canan, C., & Soares, A. L. (2019). Quality Parameters of chicken breast meat affected by carcass scalding conditions. *Asian-Australian Journal of Animal Science, 32,* 1186-1194. https://dx.doi.org/10.5713%2Fajas.18.0692

Singh, R. P., & Heldman, D.R. (1984). Introduction to Food Engineering, *Academic Press,* Orlando, FL.

Slander, J., Domingue, G., Jorgensen, F., McAlpine, K., Owen, R. J., & Bolton, F. J. (2002). Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Applied and Environmental Microbiology, 68,* 713-719. https://doi.org/10.1128/aem.68.2.713-719.2002

Sonomoto, K. & Yokota, A. (2011) Lactic Acid Bacteria and Bidifobacteria. *Caister Academic Press.*

SSD. (2021). The importance of pH in food quality and production. *Sper Scientific Direct, https://sperdirect.com/blogs/news/the-importance-of-ph-in-food-quality-and-production*

Stevenson, K.E., & Bernarnd, D.T. (1995). HACCP- Establishing Hazard Analysis Critical Control Point Programs. *The Food Processors Institute*, Washington, D.C.

Taboada, E. N., Clark, C. G., Sproston, E. L., & Carillo, C. D. (2013). Current methods for molecular typing of *Campylobacter* species. *Journal of Microbiology Methods*, 95, 24-31. https://doi.org/10.1016/j.mimet.2013.07.007

Tang, Y., Sahin, O., Pavlovic, N., LeJeune, J., Carlson, J., & Wu, Z. (2017). Rising fluoroquinolone resistance in *Campylobacter* isolated from feedlot cattle in the United States. *Scientific Reports,* 7, 494. https://doi.org/10.1038/s41598-017-00584-z

Tompkin, R.B. (1990). The use of HACCP in the production of meat and poultry products. *Journal of Food Protection, 53,* 795-803. https://doi.org/10.4315/0362-028X-53.9.795

Trezza, T. A., & Krochta, J. M. (2006). Color stability of edible coatings during prolong storage. *Journal of food science, 65*, 1166-1169. https://doi.org/10.1111/j.1365-2621.2000.tb10259.x

TTC. (2010). Overview of texture profile analysis. *Texture Technologies. https://texturetechnologies.com/resources/texture-profile-analysis*

United States Department of Agriculture. (2016). *Top 50 Ranking of Chicken Cuts.* United States Department of Agriculture. https://www.ams.usda.gov/reports/2016-top-50-ranking-chicken-cuts

United States Department of Agriculture. (2020) Broiler Chicken Industry Key Facts 2020. *National Chicken Council.* https://www.nationalchickencouncil.org/about-the-industry/statistics/broiler-chicken-industry-key-facts/

United States Department of Agriculture. (2020). Meat and Poultry HACCP. *National Agricultural Library*. https://www.nal.usda.gov/fsrio/meat-and-poultry-haccp

University of Georgia. (2020). Poultry and Eggs. *Poultry Extension*. https://extension.uga.edu/topic-areas/animal-production/poultry-eggs.html

Ustunol, Z. (2009). Edible films and coatings for meat and poultry. *Edible films and coatings for food applications, 5,* 245-268. https://doi.org/10.1007/978-0-387-92824-1_8

Vadamme P, Dewhirst F. E., Paster B. J., & On S.L. (2006). The Proteobacteria. *Bergeys's Manual of Systematic Bacteriology*, 1147-1160.

Valdes, A., Mellinas, A. C., Ramos, M., Burgos, N., Jimenez, A., & Garrigos, M. C. (2015). Use of herbs, spices and their bioactive compounds in active food packaging. *RCS Advances, 50,* 40324-40335. https://doi.org/10.1039/C4RA17286H

Valdes, A., Ramos, M., Betran, A., Jimenez, A., & Garrigos, M. C. (2017). State of the art antimicrobial edible coatings for food packaging applications. *Coatings, 56,* 1-23.

Vojdani, F., & Torres, J. A. (1990). Potassium sorbate permeability of polysaccharide films: chitosan, methylcellulose and hydrocypropyl methylcellulose. *Journal of Food Process Engineering, 12,* 33-48. https://doi.org/10.1111/j.1745-4530.1990.tb00039.x

Wehr, H. M., & Frank, J. F. (2012). Standard Methods for the Examination of Dairy Products. *American Public Health Association.*

Winther, M., & Nielsen, P. W. (2006). Active packaging of cheese with allyl isothiocyanate, an alternative to modified atmosphere packaging. *Journal of Food Protection, 69,* 2430-2435. https://doi.org/10.4315/0362-028x-69.10.2430

Whitehouse, C. A., Balbo, P. B., Pesci, E. C., Cottle, D. L., Mirabito, P. M., & Pickett, C. L. (1998). *Campylobacter jejuni* cytolethal distending toxin causes a G2-phase cell cycle block. *Infection and Immunity*, 66, 1934-1940. https://doi.org/10.1128/iai.66.5.1934-1940.1998

Wu, Y., Weller, C., Hamouz, F., Cuppett, S., & Schnepf, M. (2000). Moisture loss and lipid oxidation for precooked ground-beef patties packaged in edible starch alginate-based composite films. *Journal of Food Science, 65,* 300-304. https://doi.org/10.1111/j.1365-2621.2000.tb15997.x

Yamashita, H., Tomita, H., Inoue, T., & Ike, Y. (2011). Genetic organization and mode of action of a novel bacteriocin, determinant of VanA-type vancomycin-resistant enterococcus faecium. *Antimicrobial Agents and Chemotherapy, 55,* 4352,4360. https://dx.doi.org/10.1128%2FAAC.01274-10

Zaki, H. M., Mohamed, H. M., & El-Sherif, A.M. (2015). Improving the antimicrobial efficacy of organic acids against *Salmonella* Enterica attached to chicken skin using SDS with acceptable sensory quality. *LWT-Food Science and Technology, 64,* 558-564. http://dx.doi.org/10.1016/j.lwt.2015.06.012

Zhang, Q. Q., Han, Y. Q., Cao, J. X., Xu, X. L., Zhou, G. H., & Zhang, W. Y. (2012). The spoilage of air-packaged broiler meat during storage at normal and fluctuating storage temperatures. *Poultry Science, 91,* 208-214. https://doi.org/10.3382/ps.2011-01519

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2010). Physical and thermo-mechanical properties of whey protein isolate films containing antimicrobials, and their effect against spoilage flora of fresh beef. *Food Hydrocolloids, 24*, 49-59. https://doi.org/10.1016/j.foodhyd.2009.08.003.

CHAPTER 3

EFFECT OF A CARRAGEENAN/CHITOSAN COATING WITH ALLYL ISOTHIOCYANATE ON MICROBIAL LOAD IN CHICKEN BREAST [1]

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ABSTRACT

Each year over 20% of salmonellosis and 70% of campylobacteriosis cases are associated with poultry and poultry products in the United States. This study was aimed to screen the ability of an edible coating with Allyl Isothiocyanate (AITC) to reduce *Salmonella* Typhimurium and *Campylobacter. coli* on fresh chicken breasts by developing an edible 0.2% (w/v) Carrageenan 0.2% (w/v) Chitosan-based coating containing AITC. A high (8-7 $\log_{10}$ CFU/mL) and low (6-5 $\log_{10}$ CFU/mL) inoculum concentration was used to represent extreme and average contamination. Coating with 200 ppm of AITC reduced ($p \leq 0.05$) *Salmonella* populations by 0.98 and 1.8 $\log_{10}$ CFU/mL in high and low inoculum samples, respectively, on day 21 at 4°C. *C. coli* was reduced ($p \leq 0.05$) by 4.43 and 2.06 $\log_{10}$ CFU/mL in high and low inoculum, respectively on day 21 at 4 °C. The antimicrobial resistance of *Salmonella* Typhimurium and *C. coli* against AITC was also evaluated. It was observed that *Salmonella* exposed to 200 ppm AITC showed increased ($p \leq 0.05$) resistance to AITC after a 21-day exposure period. Similar results were not observed for *C. coli* exposed to 20 ppm of AITC. A Carrageenan/Chitosan coating with a concentration of 20 or 200 ppm of AITC reduces *Salmonella* and *C. coli* populations, thus showing potential to be used as an antimicrobial packaging material to enhance shelf life of fresh poultry.

INTRODUCTION

Chicken breasts are the most frequently bought cut of meat in the United States (USDA, 2016). With the increasing per capita consumption of chicken meat, there is a potential for an increased incidence of illness caused by *Salmonella* and *Campylobacter coli*. Each year over 20% of salmonellosis and 70% of campylobacteriosis cases are associated with poultry and poultry products in the USA (Morris Jr et al., 2011). Chicken breast contamination occurs due to cross-contamination of carcasses during processing operations and handling parts during further

processing. To reduce the prevalence of *Salmonella* and *Campylobacter* on poultry meat, the United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) has implemented new performance standards. The standards for raw chicken parts are 15.4 and 7.7% for *Campylobacter* and *Salmonella* respectively (USDA-FSIS, 2011;2016). To comply with these performance standards, poultry processors implemented antimicrobial intervention strategies during processing operations. Edible antimicrobial coatings and films are an emerging technology that has been applied to several food products ranging from meat to fruits and vegetables in the last decade (Valdes et al., 2017).

Edible coatings and films can be made from different natural polymers, including proteins, lipids, polysaccharides, and carbohydrates individually or in the composite form. The effectiveness of the material used to make the coating is closely related to its barrier property to moisture, oxygen, carbon dioxide, the characteristics of the coated product, and the storage conditions (Lin et al., 2007). Carrageenan and Chitosan are competitive candidates for forming nano-layered coatings due to their opposite electrostatic, bioactive, and non-toxic properties. Chitosan is a natural polysaccharide obtained by deacetylation of chitin and is an excellent film component due to its good oxygen barrier properties and intrinsic antimicrobial activity (Begin et al., 1999). Carrageenan is extensively used in the food industry as a gelling, emulsifying, and stabilizing agent and has been reported to have excellent film-forming properties. A combination of a Carrageenan/Chitosan coating applied using a layer by layer (up to six layers) forms a strong nanolayer coating with low interpolation due to the electrostatic interaction between the two polyelectrolytes, which is ideal for antimicrobial diffusion.

Antimicrobials commonly used in coatings include essential oils derived from plants, organic acids, bacteriocins, proteins, and polymers (Elsabee et al., 2013). Allyl Isothiocyanate

(AITC) is a volatile and aliphatic sulfur-containing compound naturally occurring in mustard seeds, that exhibits potent antimicrobial activity against Gram-Positive, Gram-Negative bacteria, and fungi (Winther, 2006). AITC is a Generally Recognized as Safe (GRAS) substance formed when glucosinolates are cleaved/hydrolyzed. Studies have shown that gaseous isothiocyanate causes metabolite leakage, measurable increases in 3-galacosidase activity, and reduction of viable bacteria by a 1,000-fold viable count at 25 ug/mL (Lin et al., 2000). Olaimat et al. (2014) reported a Carrageenan/Chitosan coating with 50 ppm of AITC reduced *Campylobacter* and *Salmonella* by 2.3 $\log_{10}$ CFU/g and <1 $\log_{10}$ CFU/g respectively after 21 days of storage at 4°C.

While the published literature has confirmed the efficacy of 50 ppm of AITC, the effectiveness of AITC at concentrations of 20 and 200 ppm against *Campylobacter* and *Salmonella* has yet to be explored. Therefore, the objective of this study was to develop a Carrageenan/Chitosan coating with AITC and apply it to chicken breasts to determine the efficacy against *Campylobacter* and *Salmonella*.

MATERIALS AND METHODS

Bacterial Strains and Inoculum Preparation

In this study, a nalidixic acid-resistant strain of *Salmonella* Typhimurium and a gentamicin-resistant strain of *Campylobacter coli* were selected. The isolates were obtained from the US National Poultry Research Center, US Department of Agricultures' Agriculture Research Station (USDA-ARS). Frozen culture of nalidixic acid-resistant *Salmonella* Typhimurium was added to 9 mL of tryptic soy broth (T.S.B.; Remel, Lenexa, KS, USA) with 200 ppm of added nalidixic acid (Sigma Aldrich, St. Louis, MO, USA**)** and incubated for 24 hours at 37°C. After incubation, cultures were transferred to two 15 ml conical tubes and centrifuged at 5500 × grams for 10 min. The pellet was re-suspended in 5 mL of phosphate-buffered saline (PBS; Fisher

Scientific, Fair Lawn, NJ, USA) and centrifuged. The supernatant was removed, and the pellet was re-suspended in 0.5 mL of PBS and added to 250 mL PBS. The final inoculum population was 8 $\log_{10}$ CFU/mL of *Salmonella Typhimurium*.

A loopful of the frozen culture of *C. coli* was streaked onto Campy-Cefex Agar (HiMedia Laboratories, Mumbai, India) supplemented with 200 ppm gentamicin (Thermo Fisher Scientific, Ward Hill, MA, US) and incubated at 42°C for 48 hours under microaerophilic conditions containing 85% N_2, 10% CO_2, and 5% O_2. The streaking process was repeated twice. After streaking, one *Campylobacter* colony from each plate was streaked for lawns onto twenty fresh Campy-cefex plates with gentamicin. The bacterial lawns were incubated, as described previously. The bacterial lawns were harvested by adding one milliliter of PBS and swabbing the bacterial lawns. The swabs were added to a 250 mL of 1% PBS. The final inoculum consisted of *C. coli* populations of ca. 6-7 $\log_{10}$ CFU/mL.

Preparation of Antimicrobial Coating

A 0.2% (w/v) Carrageenan and 0.2% (w/v) Chitosan coating (Sigma Aldrich, St. Louis, MO, USA) were prepared in 4L glass bottles. Briefly, 8 grams of Carrageenan was added to 4L of distilled water in a glass bottle and mixed for 24 hours. Eight grams of Chitosan was dissolved in 4L of 1% lactic acid solution and mixed in a glass bottle for 24 hours. The pH and temperature of the coatings were measured using a pH meter (Orion Star A111, Thermo Scientific, Ward Hill, MA, US). The coatings were placed in two separate glass containers for dipping.

Inoculation of Chicken Breasts

Fresh chicken breasts were purchased from a local grocery store and transported to the Food Safety Laboratory at the University of Georgia. Chicken breasts were used as they provided an even surface to apply coating. The chicken breasts were aseptically cut into 20 gram pieces. For

each treatment, the chicken breasts were inoculated with 1 mL of either inoculum containing *S.* Typhimurium (ca. 8 $\log_{10}$ CFU/mL), *S.* Typhimurium (ca. 5 $\log_{10}$ CFU/mL), *C. coli* (ca. 7 $\log_{10}$ CFU/mL) or *C. coli* (ca. 5-6 $\log_{10}$ CFU/mL) in a laminar flow biological safety cabinet (Labconco Corporation, Kansas City, MO, US). 0.5 mL of the inoculum was added to one side of the chicken breasts, then the breasts were flipped, and another 0.5 mL was added. The inoculated chicken breasts were placed in the biological safety hood for 15 min to allow bacterial attachment at room temperature.

Application of Antimicrobial Treatments

The inoculated samples were divided into three groups, no coating, coating, and coating + AITC. The samples were furthered divided into storage days 0, 3, 6, 9, 10, 12, 15, 18, or 21 days. The samples with no coating were put in bags (Ziplock, SC Johnson, Chicago, IL, USA) with absorbent pads (Tite-dri Industries, Boynton Beach, FL) and stored for up to 21 days at 4°C. The coating was added to the remaining samples. A newly made coating solution was used to coat the sample with *Salmonella* and *Campylobacter* to prevent cross-contamination. The inoculated samples were dipped in coatings in the following sequence Carrageenan-Chitosan-Carrageenan-Chitosan-Carrageenan to create a multilayered coating. The coating was dried in a laminar flow biological safety cabinet for 15 min and rinsed with distilled water between each layer. After the final layer of the coating was dried and rinsed, the samples with only the coating were put in bags with absorbent pads and stored at 4°C for 21 days. Allyl Isothiocyanate (AITC; Sigma-Aldrich, St Louis, MO, USA) was added to the remaining samples. The two concentrations of AITC were prepared by adding 2 mL of AITC to 98 mL of 90 proof ethanol (20 ppm of AITC) ;(Koptec, King of Prussia, PA, USA) or 20 mL of AITC to 80 mL of ethanol (200 ppm). The chicken breasts inoculated with *Salmonella* were dipped in 200 ppm of AITC, dried, rinsed, and stored at 4°C for

the appropriate length of time. The chicken breasts inoculated with *Campylobacter* were dipped in 20 ppm of AITC, dried, rinsed, and stored at 4°C. The inoculated chicken breast without the coatings served as a positive control.

Bacterial Enumeration

A sample without coating, a sample with coating, and a sample with coating + AITC in duplicate were taken out on each sampling day (0, 3, 6, 9, 12, 15, or 18) for bacterial enumeration. Each sample was placed in a sterile bag and rinsed with 20 mL of Buffer Peptone Water (BPW.; Difco, Sparks, MD, USA). Rinsing was performed for 30 sec at 300 rpm in a stomacher (Seward, 400 Circulator, England). Rinsates from each sample were collected and serially diluted in 9 mL of PBS for *Campylobacter* or 8.9 mL of PBS + 200 ppm nalidixic acid for *Salmonella.* The appropriate dilutions of *Campylobacter* samples were plated on Campy-Cefex agar supplemented with 200 ppm of gentamicin and incubated at 42°C for 48 hours under microaerophilic conditions as mentioned previously. For *Salmonella* enumeration, appropriate dilutions of *Salmonella* samples were plated on Xylose Lysine Dextrose Agar (XLD.; Himedia, Mumbai, India) and incubated at 37°C for 24 hours.

Antimicrobial Resistance

After the incubation period, a colony of either *S.* Typhimurium (low and high inoculum concentrations) or *C. coli* (low and high inoculum concentrations) was collected from the samples exposed to the Carrageenan/Chitosan coating and AITC. The colonies collected were placed in 1 mL of glycerol and stored at -80°C until further analysis. The *S.* Typhimurium samples were re-grown in XLD Agar and incubated at 37°C for 24 hours. The *C. coli* samples were re-grown in Campy-Cefex agar supplemented with 200 ppm of gentamicin and incubated at 42°C for 48 hours. The bacteria were re-streak onto new media three times. After the third pass, the bacteria were

streaked into lawns. An antimicrobial disk (Sigma-Aldrich St Louis, MO, USA) with 200 ppm of AITC was placed over the *S.* Typhimurium microbial lawn and stored at 37 °C for 24 hours. An antimicrobial disk with 20 ppm of AITC was placed over the *C. coli* microbial lawn and stored at 42°C for 48 hours. After the incubation period the zone of inhibitions were measured.

Statistical Analysis

The experiment was performed as three independent replications for each treatment combination on a particular day. For each replication, fresh inoculum, Carrageenan/Chitosan coating, and AITC solutions were prepared on the day of the experiment, and fresh chicken breasts were purchased. The data were analyzed using analysis of variance (ANOVA) in the General Linear Model (GLM) of SAS. (SAS 9.4 Institute, Inc., Cary, NC, US). Statistical differences between the treatments were reported as least-square means, and significance was reported at a level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of an Edible Antimicrobial Coting against low and high inoculum concentration of *Salmonella* Typhimurium

The initial population of *S.* Typhimurium on the chicken breasts (positive control) inoculated with a high concentration of inoculum was 7.57 $\log_{10}$ CFU/mL (Figure 1) whereas the initial population of *S.* Typhimurium on the chicken breast (positive control) inoculated with a low inoculum concentration was 5.26 $\log_{10}$ CFU/mL (Figure 2). The concentration ($\log_{10}$ CFU/mL) of *Salmonella* decreased over 21 days of storage in all treatments and inoculum concentrations ($p \leq 0.05$). There was no significant ($p > 0.05$) reduction observed on samples with Carrageenan/Chitosan coating on each sampling day when compared to samples with no coating (positive control). In contrast a study conducted by Olaimat et al. (2014), a coating containing

0.2% Chitosan and 0.2% K-carrageenan significantly reduced numbers of *Salmonella* on chicken breast by 0.8 $\log_{10}$ CFU/g at 4°C. This can be potentially explained by Oliamat's study were vacuumed packaged before they were stored thus contributed to the reduction compared to the current study. Another study conducted by Chen et al. (2012) also that reported a 0.2% chitosan coating reduced *Salmonella* numbers on cantaloupe 1.5 $\log_{10}$ CFU/cm^2 after 24 hours at 22°C. The current study may differ because it was a long-term storage study on a different product. However, Upadhyaya et al. (2015) reported that a pectin coating did not significantly reduce *S.* Enteritidis, and a chitosan coating did not significantly reduce *L. monocytogenes* on shell eggs.

When in combination with 200ppm AITC, 0.2% Carrageenan and 0.2% Chitosan coating caused a reduction ($p \leq 0.05$) of 1.51, 1.41, 1.66 1.45, 1.33, 1.09, 0.73 and 0.98 $\log_{10}$ CFU/mL over the 21 days at 4°C storage period (sampled every 3 days) in samples inoculated with high levels of inoculum. Samples with low inoculum, 0.2% Chitosan and 0.2% Carrageenan plus AITC resulted in reductions ($p \leq 0.05$) of 1, 1.24, 1.16, 1.01, 1.78, 1.9, 1.7 and 1.8 $\log_{10}$ CFU/mL over the 21 days at 4°C storage period, sampled every 3 days. AITC at 200 ppm was shown to be the minimum bactericidal concentration (MBC) because it resulted in a 1,000-fold reduction in bacterial population at 24 hours in both samples with a low and high inoculum concentration. Chen et al. (2012) also reported that incorporating 60 µL/g AITC reduced *Salmonella* on cantaloupe surfaces by > 5 $\log_{10}$ CFU/mL after 24 hours at 22°C. Jin et al. (2013) reported releasing AITC vapor at 1.2 µg/h reduced *S.* Typhimurium in modified atmosphere packaged (MAP) chicken breasts by 1.3 $\log_{10}$ CFU/mL after 21 days in storage. Additionally, Olaimat et al. (2014) concluded that 50 µL AITC/g reduced the numbers of *Salmonella* on vacuum-packed chicken breasts 2.3 $\log_{10}$ CFU/g by day 21 at 4°C. The antimicrobial properties of AITC on *Salmonella* may be due to the three possible action mechanisms. First, AITC may disrupt the permeability of the cell

membrane (Zou et al., 2013). Second, AITC may disrupt *Salmonella's* energy mechanisms (Chan et al., 2013). Lastly, AITC may disrupt disulfide bonds and cause oxidative cleavage leading to enzymatic inactivation (Luciano et al., 2009). The chitosan coating may also provide some antimicrobial activity due to its ability to change cell permeability by its interaction with electronegative charges on the bacteria cell (Martinez-Camacho et al., 2010). Chitosan may also interact with DNA and interfere with messenger RNA synthesis (Rabea et al., 2013). Moreover, Chitosan can inhibit microbial growth because it is a chelating agent that binds to essential metals (Tripathi et al., 2010).

Antimicrobial activity of Edible Antimicrobial Coating against low and high inoculum concentration of *C. coli*

The initial *C. coli* population on chicken breasts with a high inoculum concentration (positive control) was 7-8 $\log_{10}$ CFU/mL (Figure 3). The initial *C. coli* population on chicken breasts with a low inoculum concentration (positive control) was 5-6 $\log_{10}$ CFU/mL (Figure 4). The concentration ($\log_{10}$ CFU/mL) of *C. coli* decreased over 21 days of storage in all treatments and inoculum concentrations ($p \leq 0.05$). There was no significant reduction observed on samples with Carrageenan/Chitosan coating on each sampling day when compared to samples with no coating (positive control). In contrast Wagle et al. (2018) stated that a pectin coating present in poultry products during storage could potentially protect the product from microbial contamination during handling and further processing. Additionally, Wagle et al. (2018) concluded that a chitosan coating could significantly reduce the presence of *C.coli* over seven days in vacuum-packed chicken. However, the study concluded that adding the antimicrobial eugenol increased the antimicrobial reduction of the coating.

When in combination with 20ppm AITC, 0.2% Carrageenan and 0.2% Chitosan coating caused a reduction ($p \leq 0.05$) of 0.77, 2.17, 2.56, 3.55, 3.37, 3.26, 2.06 and 2.21 $\log_{10}$ CFU/mL over the 21 days storage period (samples every 3 days) in samples inoculated with high levels of inoculum. Samples with low *C. coli* inoculum, 0.2% Chitosan and 0.2% Carrageenan plus AITC resulted in reductions of 2.22, 2.77, 3.19, 3.71, 4.24, 4.43, 4.43 and 4.43 $\log_{10}$ CFU/mL ($p \leq 0.05$) in each sampling day respectively for 21 days at 4°C. AITC at, 20 ppm was shown to be the minimum bactericidal concentration (MBC) for samples with a low *C. coli* inoculum at 24 hours. It resulted in a 1,000-fold reduction in bacterial population at 24 hours. In samples with a high *C. coli* inoculum 20 ppm of AITC resulted in the minimum bactericidal concentration (MBC) at 48 hours. Olaimat et al. (2014) reported that a K-carrageenan/chitosan solution containing 50 or 100 ul/g AITC reduced *C. jejuni* to an undetectable level (<1.0 $\log_{10}$ CFU/g) after five days of storage. Other studies have explored the effects of other antimicrobials in combination with pectin/chitosan treatments. Wagle et al. (2018) reported that incorporating eugenol in a coating material significantly improved the efficacy of pectin and chitosan coating material in poultry products. Previous studies have determined that phytochemicals such as AITC and eugenol at subinhibitory concentration change the gene expression of several microorganisms, including *C. coli and C. jejuni* (Arambel et al. 2015, Upadyay et al. 2017, Wagle et al. 2014, Castillo et al. 2014, Oh et al. 2015, Kovacs et al. 2016). A study performed by Wagle et al. (2018) characterized the genes affected by eugenol and determined that the phytochemical significantly altered the gene expression of motility (*motA, motB)* and quorum sensing (*luxS)* in chicken meat juice. Additionally, eugenol also downregulated the stress response gene *katA.*

Antimicrobial Resistance of *Salmonella.* Typhimurium and *C. coli* against AITC.

The current experiment also tested the antimicrobial resistance developed towards AITC over 21 days of *C. coli* and *Salmonella* Typhimurium. The zone of inhibition of AITC was significantly reduced ($p \leq 0.05$) in *Salmonella* Typhimurium samples that were previously exposed to 200 ppm of AITC for over 21 days. The zone of inhibition of AITC in *C. coli* samples exposed to AITC for 21 days did not change significantly. This may be due to the different mechanisms of action of AITC in *Salmonella* and *C. coli* previously mentioned. Additionally, the higher concentration of 200 ppm used against *Salmonella* compared to the lower concentration of 20 ppm used against *C. coli* might have contributed to the development of antimicrobial resistance. A review by AlSheikh et al. (2020) on plant-based phytochemicals as an alternative to antibiotics in combating bacterial drug resistance concluded that to properly address the feasibility of phytochemicals, there needs to be a further exploration of their efficacy without causing resistance.

LIST OF TABLES AND FIGURES

Table 1

Day	*Salmonella* Low Inoculum	*Salmonella* High Inoculum	*Campylobacter* Low Inoculum	*Campylobacter* High Inoculum
0	4.6 ± 0.46^{A}	4.6 ± 0.15^{A}	4.6 ± 0.76^{A}	5.8 ± 0.12^{A}
3	3.8 ± 0.51^{B}	4 ± 0.1^{AB}	5.2 ± 0.17^{A}	5.4 ± 0.29^{A}
6	3.6 ± 0.42^{BC}	3.6 ± 0.15^{AB}	5.2 ± 0.36^{A}	4.8 ± 0.12^{A}
9	3.6 ± 0.31^{CD}	3.2 ± 0.17^{AB}	5.6 ± 0.29^{A}	5.2 ± 0.36^{A}
12	3.2 ± 0.21^{D}	2.8 ± 0.2^{B}	4.8 ± 0.55^{A}	4.8 ± 0.57^{A}
15	3.0 ± 0^{D}	2.8 ± 0.1^{B}	4.4 ± 0.57^{A}	4.8 ± 1.06^{A}
18	3.0 ± 0^{D}	2.8 ± 0^{B}	4.6 ± 0^{A}	5.6 ± 0^{A}

Figure 1

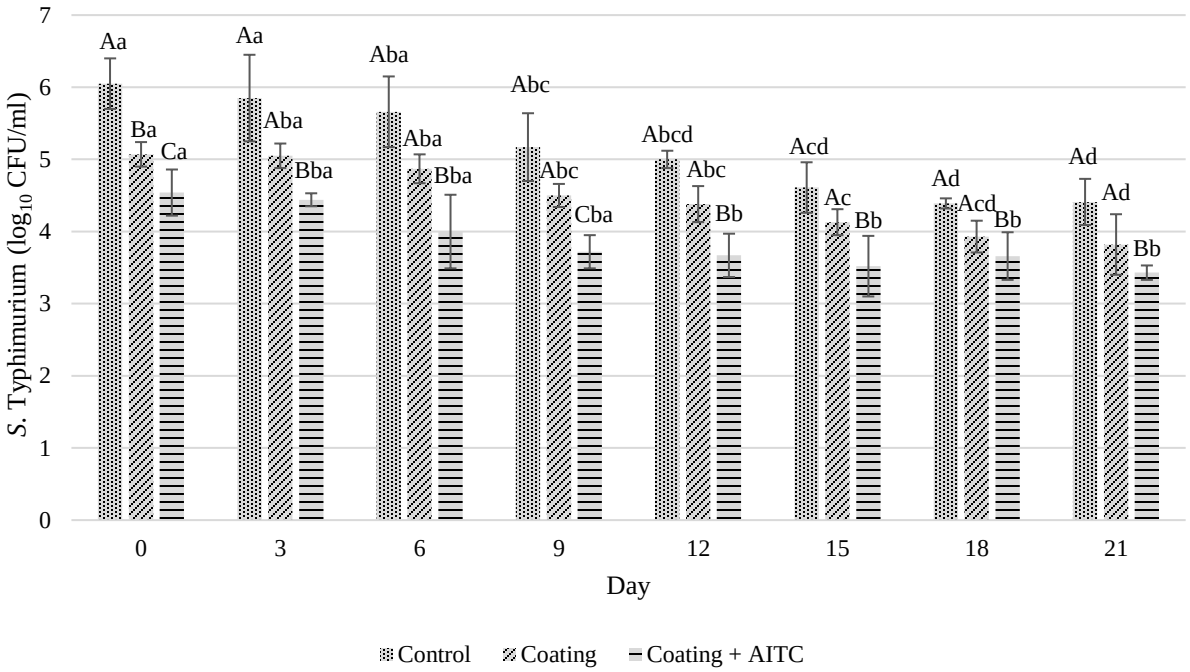

7
6
5
4
3
2
1
0
S. Typhimurium (log10 CFU/ml)
Aa
Ba
Ca
Aa
Aba
Bba
Aba
Aba
Bba
Abc
Abc
Cba
Abcd
Abc
Bb
Acd
Ac
Bb
Ad
Acd
Bb
Ad
Ad
Bb
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + AITC

Figure 2

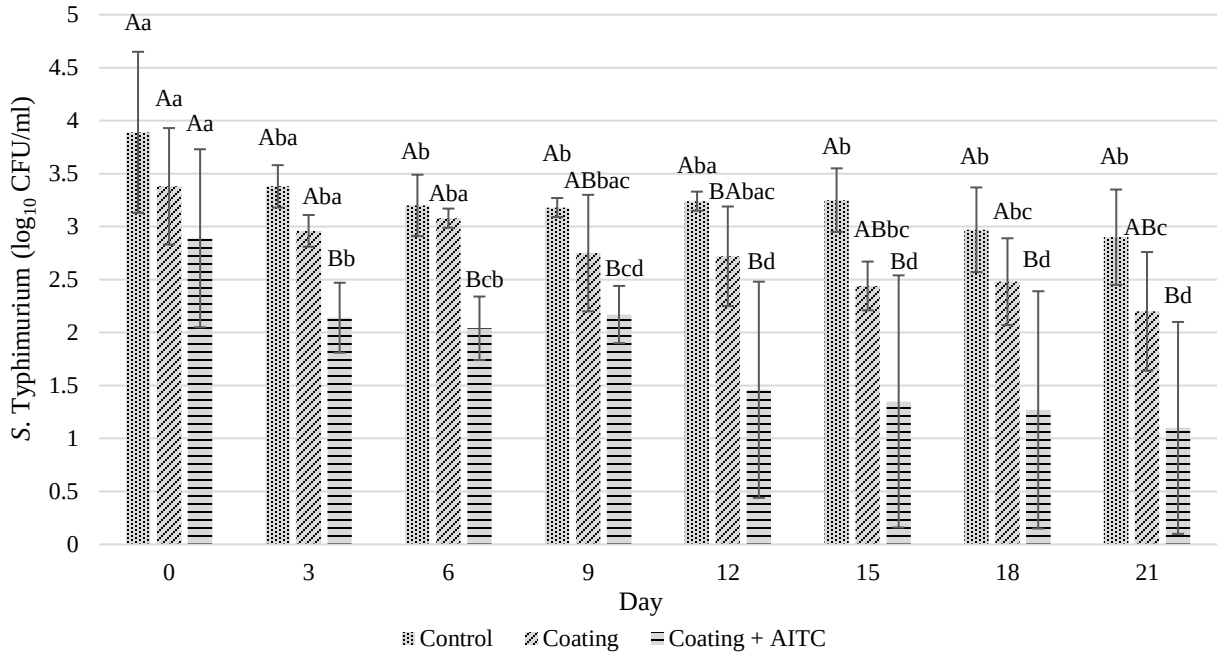

S. Typhimurium (log10 CFU/ml)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Aa
Aa
Aa
Aba
Aba
Bb
Ab
Aba
Bcb
Ab
ABbac
Bcd
Aba
BAbac
Bd
Ab
ABbc
Bd
Ab
Abc
Bd
Ab
ABc
Bd
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + AITC

Figure 3

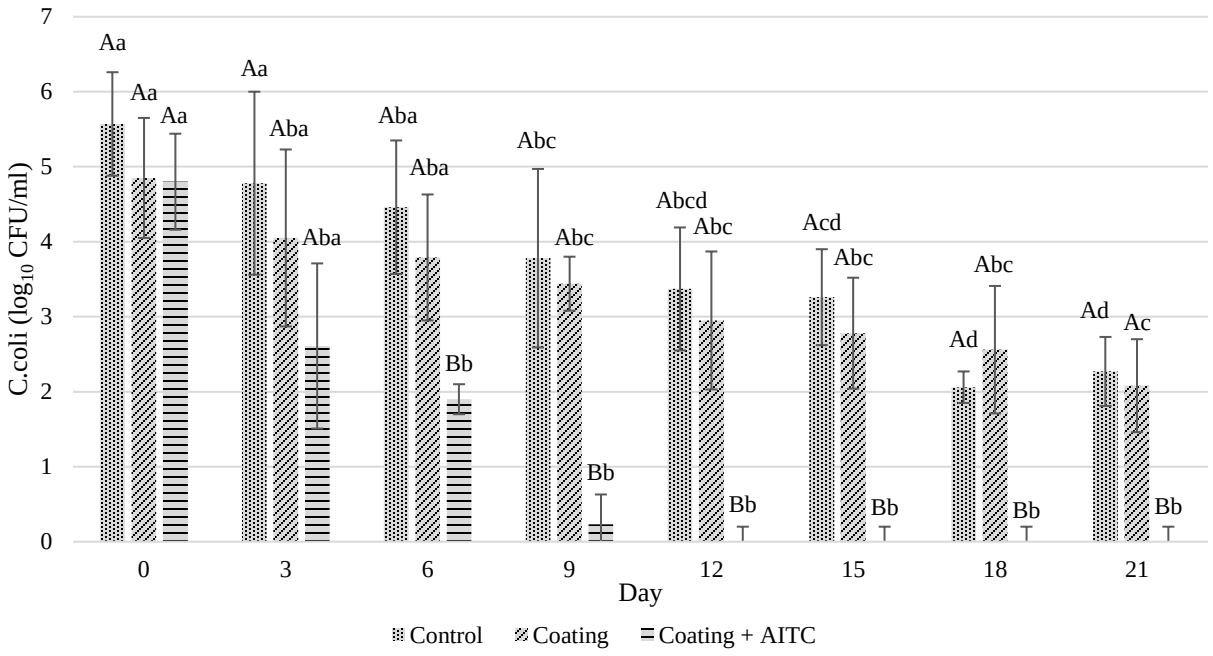
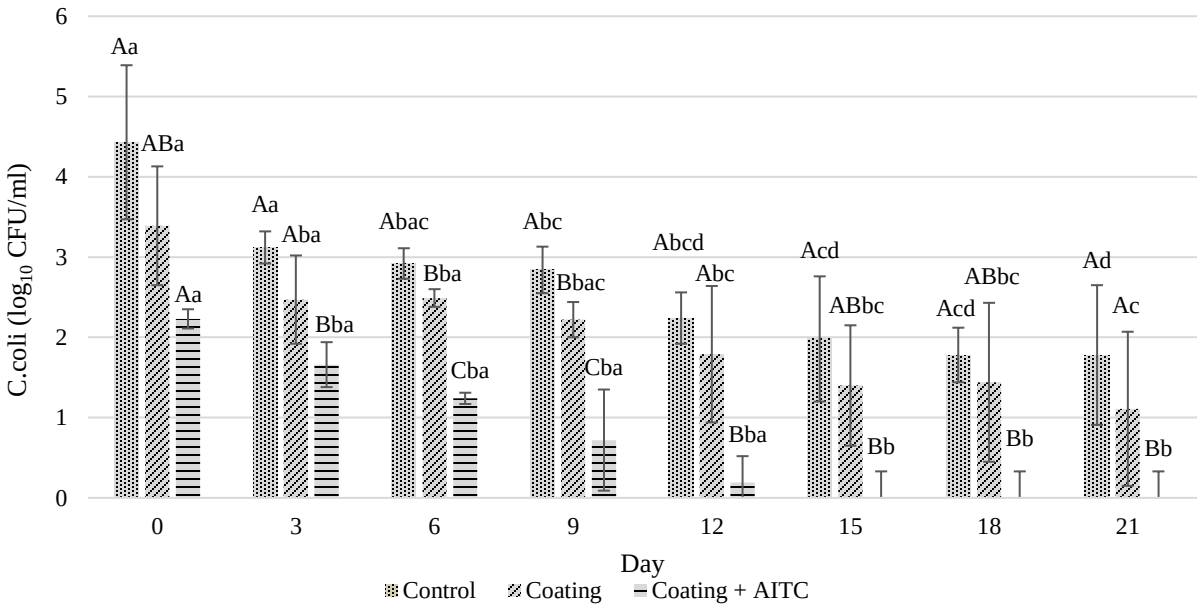

7
6
5
4
3
2
1
0
C.coli (log10 CFU/ml)
Aa
Aa
Aa
Aa
Aba
Aba
Aba
Aba
Bb
Abc
Abc
Bb
Abcd
Abc
Bb
Acd
Abc
Bb
Ad
Abc
Bb
Ad
Ac
Bb
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + AITC

Figure 4


C.coli (log10 CFU/ml)
6
5
4
3
2
1
0
Aa
ABa
Aa
Aa
Aba
Bba
Abac
Bba
Cba
Abc
Bbac
Cba
Abcd
Abc
Bba
Acd
ABbc
Bb
Acd
ABbc
Bb
Ad
Ac
Bb
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + AITC

REFERENCES

AlSheikh, H. M. A., Sultan, I., Kumar, V., Rather, I. A., Al-Sheikh, H., Jan, A. T., & Haq, Q. M. R. (2020) Plan-Based Phytochemicls as Possible Alternative to Antibiotics in Combating Bacterial Drug Resistance. *Antibiotics, 9,* 480. https://dx.doi.org/10.3390%2Fantibiotics9080480

Arambel, H. R., Donoghue, A. M., Arsi, K., Upadhyay A., Woo-Ming, A., Blore, P. J., Venkitanarayanan, K., & Donoghue, D. J. (2015). Chitosan supplementation reduces enteric colonization of *Campylobacter jejuni* in broiler chickens and down-regulates expression of colonization genes. *Advance Food Technology Nutitional Science, 1,* 104-111. http://dx.doi.org/10.17140/AFTNSOJ-119

Begin, A., & Van Calsteren, M. R. (1999). Antimicrobial films produced from chitosan. *International Journal of Biological Macromolecules, 26,* 63-67. https://doi.org/10.1016/s0141-8130(99)00064-1

Casitillo, S., Heredia, N., Arechiga-Carvajal, E., & Garcia, S. (2014). Citrus extracts as inhibitors of quorum sensing, biofilm formation and motility of *Campylobacter jejuni. Food Biotechnology, 28,* 106-122.

Chen, W. X., Jin, T. Z., Gurtler, J. B., Geveke, D. J., & Fan, X. (2012). Inactivation of *Salmonella* on whole cantaloupe by application on an antimicrobial coating containing chitosan and allyl isothiocyanate. *International Journal of Food Microbiology, 155,* 165-170. https://doi.org/10.1016/j.ijfoodmicro.2012.02.001

Elsabee, M. Z., & Abdou, E. S. (2013). Chitosan based edible films and coatings. *Material Science and Engineering, 33,* 1819-1841. https://doi.org/10.1016/j.msec.2013.01.010

FAO. (2021). *Overview of global meat market developments in 2020*. Food and Agriculture Organization of the United Nations Meat Market Review. http://www.fao.org/publications/card/en/c/CA8819EN/

Jin, T. Z., Gurtler, J. B., & Li, S. Q. (2013). Development of antimicrobial coating for the microbiological safety and quality of shell eggs. *Journal of Food Protection, 76,* 779-785. https://doi.org/10.4315/0362-028x.jfp-12-460

Kovacs, J. K., Felso, P., Makszins, L., Papai, Z., Horvath, G., Abraham, H., Palkovics, T., Bozormenyi, A., Emody, L., & Schneider, G. (2016). Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen *Campylobacter jejuni. Applied Environmental Microbiology,* 82, 615-6166.

Lin, C. M., Preston, J. F., & Wei, C. I. (2000). Antimicrobial mechanism of allyl isothiocyanate. *Journal of Food Protection, 63,* 727-734. https://doi.org/10.4315/0362-028x-63.6.727

Lin, D., & Zhao, Y. (2007). Innovations in the development and applications of edible coatings for fresh and minimally processed fruits and vegetables. *Comprehensive Reviews in Food Science and Food Safety, 6,* 60-75. https://doi.org/10.1111/j.1541-4337.2007.00018.x

Luciano, F. B., & Holley, R. A. (2010). Bacterial degradation of glucosinolates and its influence on the growth of *E. coli* O157:H7. *Fleischwirtschaft International,* 26, 78-71.

Martinez-Camacho, A. P., Cortez-Rocha, M. O., Ezquerra-Brauer, J. M., Graciano-Verdugo, A. Z., Rodriguez-Felix, F., Castillo-Ortega, M. M., Yepz-Gomez, M. S., & Plascencia-Jatomea, M. (2010). Chitosan composite films: thermal, structural mechanical and antifungal properties. *Carbohydrates and polymers, 82,* 305-315. https://doi.org/10.1016/j.carbpol.2010.04.069

Morris, J. G., Hoffman, S., & Batz, B. (2011). Ranking risks: the 10 pathogen-food combinations with the greatest burden on public health. *Purdue University*. http://hdl.handle.net/10244/1022

Oh, E., & Jeon, B. (2015). Synergistic anti-*Campylobacter jejuni* activity of fluoroquinolone and macrolide antibiotics with phenolic compounds. *Frontiers in Microbiology*, 6, 1129. https://doi.org/10.3389/fmicb.2015.01129

Olaimat, A. N., Fang, Y., & Holley, R. A. (2014). Inhibition of *Campylobacter jejuni* on fresh chicken breasts by k-carrageenan/chitosan-based coatings containing allyl isothiocyanate or deodorized oriental mustard extract. *International Journal of Food Microbiology, 187,* 77-82. https://doi.org/10.1016/j.ijfoodmicro.2014.07.003

Olaimat, A. N., & Holley, R. A. (2015). Control of *Salmonella* on fresh chicken breast by k-carrageenan/chitosan-based coatings containing allyl isothiocyanate or deodorized oriental mustard extract plus EDTA. *International Journal of Food Microbiology, 48,* 83-88. https://doi.org/10.1016/j.fm.2014.11.019

Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G., & Steubaut, W. (2003). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules, 4*, 1457-1465. https://doi.org/10.1021/bm034130m

Rojas-Grau, M. A., Solica-Fortuny, R., & Martin-Belloso, O. (2009). Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. *Trends Food Science and Technology, 20*, 438-447. https://doi.org/10.1016/j.tifs.2009.05.002

Tripathi, S., Mehrotra, G. K., & Dutta, P. K. (2010). Preparation and physicochemical evaluation of chitosan/poly(vinyl alcohol)/pectin ternary film for food-packing applications. *Carbohydrates Polymers, 79,* 711-716. https://doi.org/10.1016/j.carbpol.2009.09.029

Upadhyaya, I., Yin, H. B., Nair, M. S., Chen, C. H., Lang, R., Darre, M. J., & Venkitanarayanan, K. (2016). Inactivation of *Salmonella Enteritidis* on shell eggs by coating with phytochemicals. *Poultry Science,* 95, 2106-2111. https://doi.org/10.3382/ps/pew152

USDA. (2011). New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishment. *Federal Registry*, 76, 15282-15290.

USDA. (2016). Top 50 Ranking of Chicken Cuts. *United States Department of Agriculture.*

Valdes, A., Ramos, M., Sanahuja, A. B., & Jimenez, A. (2017). State of the art of antimicrobial edible coatings for food packaging applications. *Coatings, 7*. http://dx.doi.org/10.3390/coatings7040056

Wagle, B. R., Arsi, K., Upadhyay, A., Shrestha, S., Venkitanarayanan, K., Donoghue, A. M., & Donoghue, D. J. (2017). Beta-resorcylic acid, a phytophenolic compound, reduces *Campylobacter jejuni* in postharvest poultry. *Journal Food Protection, 80,* 1243-1251. https://doi.org/10.4315/0362-028x.jfp-16-475

Wagle, B. R., Upadhyay, A., Shrestha, S., Arsi, K., Upadhyaya, I., Donoghue, A. M., & Donoghue, D. J. (2019). Pectin or chitosan coating fortified with eugenol reduces *Campylobacter jejuni* on chicken wingettes and modulates expression of critical survival genes. *Poultry Science, 98*, 1462-1471. https://doi.org/10.3382/ps/pey505

Winther, M., & Nielsen, P. W. (2006). Active packaging of cheese with allyl isothiocyanate, an alternative to modified atmosphere packaging. *Journal of Food Protection, 69*, 2430-2435. https://doi.org/10.4315/0362-028x-69.10.2430

Zou, Y., Jung, L. S., Lee, S. H., Kim, S., Cho, Y., & Ahn, J. (2013) Enhanced antimicrobial activity of nisin in combination with allyl isothiocyanate against *Listeria monocytogenes*,

Staphylococcus aureus, Salmonella Typhimurium and *Shigella boydii*. *International Journal Food Science Technology*, *48*, 324-333. https://doi.org/10.1111/j.1365-2621.2012.03190.x

CHAPTER 4

EFFECT OF A CARRAGEENAN/CHITOSAN COATING WITH ALLYL ISOTHIOCYANATE ON SPOILAGE LOAD AND QUALITY OF CHICKEN BREAST[1]

[1]Moller, A. E. To be submitted to Journal of Poultry Science

ABSTRACT

A minimum of 3.7% of poultry meat is lost due to spoilage each year in the United States. This study was aimed to screen the ability of a Carrageenan/Chitosan coating with Allyl Isothiocyanate (AITC) to reduce lactic acid bacteria (LAB), aerobic bacteria, yeast, molds, and rancidity without affecting color and pH. The concentrations tested in this experiment were 20 and 200 ppm of AITC. Coating with 20 and 200 ppm of AITC consistently reduced ($p \leq 0.05$) yeast and molds by at least 1.77 $\log_{10}$ CFU/ml from day 3 to 21 at 4 °C compared to untreated control. Coating with 20 and 200 ppm of AITC increased the time before aerobic spoilage occurs by 3 and 6 days, respectively compared to untreated control. Coating 20 and 200 ppm of AITC increased the time before lactic acid bacteria spoilage by at least 3 days compared to the control. Additionally, 20 and 200 ppm of AITC significantly alter ($p \leq 0.05$) the color by increasing the lightness and making the meat less red and yellower over 21 days at 4 °C. The pH in the untreated control was also significantly higher ($p \leq 0.05$) the samples with 20 or 200 ppm of AITC over 21 days at 4 °C. The edible antimicrobial coating did not significantly reduce the rancidity over a 21-days storage period. A Carrageenan/Chitosan coating with a concentration of 200 ppm of AITC reduces spoilage aerobic bacteria, yeast and molds and LAB populations, thus showing potential to be used as an antimicrobial packaging material to enhance shelf life of fresh poultry.

INTRODUCTION

The United States is the world's top poultry producer in the world (USDA, 2020). The poultry consumption in the US has doubled since 1978. Out of all poultry cuts, chicken breast is the most frequently consumed cut of meat (USDA, 2016). However, with the increase in production and consumption, it is estimated that between 3.7 and 4.2% of poultry meat is lost due to spoilage each year (Buzby et al., 2009). Spoilage of meat may be due to prolonged storage time,

improper storage temperature, contamination, or high ph. Food spoilage causes a decrease in quality because it produces off-flavors, off-colors, off-odors, and microbial growth (Remington, 2017). To prevent changes in quality, poultry processors are looking for emerging technologies to reduce spoilage. Previously, techniques such as heat treatment, salting or acidification, have been applied in the food industry for years to reduce spoilage (Lucera et al., 2012). Edible coatings and films serve as an alternative emerging technology to increase shelf life in products.

Edible films and coating can be made of proteins, polysaccharides, lipids, and carbohydrates individually or in the composite. Coatings can control gas exchange, moisture permeation, gas permeation, or oxidation. Carrageenan and Chitosan are good candidates for coatings due to their ability to form multilayered coatings with opposite electrostatic charges. Chitosan is a catanionic polysaccharide obtained by deacetylation of chitin. It is a good candidate for coatings due to its oxygen barrier properties, antimicrobial activity, and antioxidant activity (Xia, 2011). Carrageenan a sulfated anionic polysaccharide, has been extensively used in the food industry as a gelling and stabilizing agent (Yegappan et al., 2018). Forming a Carrageenan/Chitosan coating using the layer-by-layer technique for a total of six layers forms a coating in which an antimicrobial can attach to and diffuse.

The antimicrobial Allyl Isothiocyanate (AITC) is a volatile and aliphatic sulfur-containing compound extracted from mustard seeds used in coatings. AITC is a GRAS substance, and it is the primary flavor component in many foods such as wasabi, horseradish, and mustard. Gaseous AITC causes metabolite leakage and a threefold increase in galactosidase activity which causes a reduction in Gram-Positive, Gram-Negative bacteria and fungi (Winther, 2016). The use of AITC in food systems is limited due to its high volatility and strong odor (Ko et al., 2012). A study conducted on *Kimchi* concluded that adding AITC can increase pH, lower titratable acidity,

decrease spoilage bacteria, and improve texture. However, the overall scores of acceptability decreased due to the odor of AITC. The study concluded that AITC is an effective way of enhancing the shelf-life of *Kimchi* without reducing quality (Ko et al., 2012).

Although previous studies have found AITC/Chitosan coatings effective on Kimchi, similar studies have yet to be conducted on chicken. Therefore, the objective of this study was to develop a Carrageenan/Chitosan coating with 20 or 200 ppm of AITC and apply it to chicken breasts to determine its efficacy against spoilage bacteria without affecting the quality parameters: color, texture, pH, and rancidity.

MATERIALS AND METHODS

Preparation of Antimicrobial Coating

A 2% (w/v) Carrageenan and 2% (w/v) Chitosan (Sigma Aldrich, St. Louis, MO, US) solution were prepared separately in 4 L glass bottles. Briefly, 8g of Chitosan was added to 4 L of distilled water in a glass bottled and mixed for 24 hours. Eight grams of chitosan were dissolved in 4 L of 1% lactic acid solution and mixed in a glass bottler for 24 hours. The pH and temperatures were measured using a pH meter (Orion Star A111, Thermo Scientific, Ward Hill, MA, USA). The coatings were places in two separate glad containers for dipping.

Application of Antimicrobial Treatments

Fresh chicken breasts were purchased from a local grocery store and transported to the Food Safety Laboratory at the University of Georgia. The chicken breasts were aseptically cut into 20-gram pieces. The chicken breasts were divided into two groups, samples that were going to be tested for quality, or samples tested for spoilage. The samples were then divided into four categories, coating + 20 ppm AITC, coating + 200 ppm AITC, coating, no coating. The samples were furthered divided into storage days 0, 3, 6, 9, 12, 15, 18 and, 21 days. The samples with no

coating were put in bags (Ziplock, SC Johnson, Chicago, IL, USA.) with absorbent pads (Tite-dri Industries, Boynton, Beach, FL) and stored for up to 21 days at 4 °C. The remaining samples were dipped in coatings in the following sequence Carrageenan-Chitosan-Carrageenan-Chitosan-Carrageenan to create a multilayer coating. In between each layer the coating was dried in a laminar flow biological safety cabinet for 15 min and rinsed with sterile distilled water. After the last layer, the samples were dried and rinsed. Samples with only the coating were stored for the appropriate amount of time at 4 °C. The remaining samples were then dipped in either 20 or 200 ppm of AITC and stored as mentioned previously. The chicken breast with no coating served as a positive control.

Spoilage Bacteria Enumeration

A sample without coating, sample with coating, sample with coating + 20 ppm AITC and a sample with 200 ppm AITC in duplicate were taken out on each sampling day (0, 3, 6, 9, 12, 15, 18 or 21) for spoilage enumeration. The chicken breasts were placed in sterile bags and rinsed with 20 mL Buffered Peptone Water (BPW; Difco, Sparks, MD, US). Rinsing was performed for 30 sec at 300 rpm in a stomacher (Seward, 400 Circulator, England). The rinsate was collected and serially diluted in 9 mL of PBS. The appropriate dilutions were plated in Lactic Acid Bacteria Count Plates, Rapid Aerobic Count Plates, and Yeast and Mold Count Plates (3M Petrifilms, St. Paul, MN, USA). The Rapid Aerobic Count Plates and Lactic Acid Bacteria Count Plates were stored at 37 °C for 24 hours and 48 hours, respectively. Yeast and Mold Count Plates were stored at 25 °C for 72 hours.

Quality Analysis

A sample without coating, coating, coating + 20 ppm AITC and coating + 200 ppm AITC in duplicate were taken out each sampling day (0, 3, 6, 9, 12, 15, 18, or 21) for quality analysis.

The color was measured according to the CIE-L*a*b* system (Chroma Meter CR-400, Konica Minolta, Japan) on the surface of the chicken breast. The pH was measured on the surface of the chicken breast using a surface pH meter (HQ11d, HACH, USA). The rancidity was tested by blending 10-grams of chicken breast with deionized water in a blender (The Original, Magic Bullet, China) for 2 min. Two milliliters of the homogenate were combined with 4 mL of TCA/TBA reagent and 100 µL of BHA and vortex thoroughly. The solution was heated for 15 min, cooled for 10 min, vortex thoroughly, and centrifuged at 2000 g for 10 min. The absorbance of the supernatant at 531 nm was read. The absorbance was compared to a TEP standard curve and the concentration of TBAR (ug/mL) was determined.

Statistical Analysis

The experiment was performed in triplicate for each treatment combination on separate days. For each replication, Carrageenan/Chitosan coating and AITC solutions were prepared on the day of the experiment, and fresh chicken breasts were purchased on the day of production. The data were analyzed using analysis of variance (ANOVA) in General Linear Model (GLM) of SAS (SAS 9.4 Institute, Inc., Cary, NC, USA). Statistical differences between the treatments were reported as least square means and significance was reported at a level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of coatings against aerobic, lactic acids bacteria, yeasts, and molds

Figures 1, 2, and 3 show the effect of a Carrageenan/Chitosan coating alone or with either 20 or 200 ppm of Allyl Isothiocyanate (AITC) on aerobic bacteria, lactic acid bacteria (LAB), and yeast/mold on chicken breasts. According to Gill (2003) aerobic bacteria spoilage occurs when bacteria numbers reach 6 $\log_{10}$ CFU/mL. Additionally, LAB spoilage occurs when bacteria numbers reach 8 $\log_{10}$ CFU/mL. In this study aerobic bacteria increased in all treatments over the

21-day storage period at 4°C. Samples with no coating (positive control) reached aerobic spoilage on day 6 in contrast samples with coating, coating + 20 ppm and coating + 200 ppm reached aerobic spoilage on days 9, 12 and 15, respectively. Lactic acid bacteria population also increased in all samples over time. However, samples with no coating (positive control) reached LAB spoilage on day 15 in contrast samples with coating and coating + 20 reached bacteria LAB spoilage on day 18. Samples with coating +200 ppm of AITC did not reached LAB spoilage by the end of the experiment on day 21. The standard yeast and mold spoilage concentrations are not known. However, in this study the yeast and mold populations increased in all treatments over the 21-day storage period at 4°C. All tested doses of AITC consistently reduced ($p \leq 0.05$) yeast/mold by at least 1.77 $\log_{10}$ CFU/mL respectively from day 3 to day 21 compared to the untreated control.

These bacteria were explored because they commonly decrease the shelf life of products. A previous study concluded that coating chicken wings with the phytochemical eugenol and its coating materials significantly reduced total aerobic counts (Kim et al., 2000). Wagle et al. (2019) also reported that eugenol significantly reduced the aerobic bacteria count by at least 0.51 $\log_{10}$ CFU/mL. A study conducted by Olaimat et al. (2015) reported that 50 µL/mL of AITC reduced LAB by 3.0 $\log_{10}$ CFU/mL at day 21. It has been reported that LAB starter cultures and adventitious LAB in meat were more resistant to AITC than *Salmonella* (Olaimat et al. 2015). Oliamat et al. (2014) concluded that this could be due the different effect of AITC on gram-negative and gram-positive bacteria. This is similar to the results of this experiment which reported that LAB is more resistant to AITC than aerobic bacteria. A review done on the effect of phytochemicals in antifungal bioactivity concluded that several phytochemicals have an antifungal mechanism which includes inhibition of cellular membrane biosynthesis, alteration of cellular membrane permeability or reactivity with proteins thiol-moieties, all of which cause a reduction

in fungal fitness, cell death, or both (Redondo-Blanco, 2020). From the present work, it is evident that the coating with 20 or 200 ppm of AITC reduced the number of spoilage microorganisms, and this may enhance the quality of chicken and extend its shelf life.

Effect of Antimicrobial Coating on Color of Chicken Breasts

Table 1 shows the effect of a Carrageenan/Chitosan coating alone or in combination with either 20 or 200 ppm of Allyl Isothiocyanate on the lightness, greenness, and yellowness of chicken breasts. The Chitosan/Carrageenan coating alone did not significantly affect the color of chicken breast. However, applying a coating with either 20 or 200 ppm of AITC significantly increased the lightness of the chicken breast ($p< 0.05$) by day 9 through day 21 (Table 1). Additionally, samples with 200 ppm of AITC became greener compared to other treatments ($p<0.05$). The meat also became yellower over 21 days when the coating with either concentration of AITC was applied to the chicken breasts ($p<0.05$).

Similar, to the results of this study, Wagle et al. 2019 reported that a significant change in color was not observed in chicken wingettes during a short storage period. However, during long storage periods, Khan et al. (2015) reported that chicken with the phytochemical eugenol stored for six days became lighter and more brownish. Wagle et al. 2019 also reported that the chicken becomes yellower, probably because of the coating in combination with eugenol over long storage periods.

Effect of Antimicrobial Coating on the pH of Chicken Breasts

Figure 8 shows the effect of a Carrageenan/Chitosan coating alone or with either 20 or 200 ppm of AITC on the pH of chicken breasts. The initial pH of the chicken breast without coating (positive control) was 5.73. After 21 days of storage, the pH increased significantly to 6.65. The

pH remained constant in samples with Carrageenan/Chitosan coating alone or with either 20 or 200 ppm of AITC.

These results are similar to a previous study conducted by Wu et al. (2014) that concluded that the control group had a higher pH value than the treatment group with a Chitosan/Chitooligosaccharides/Glutathione coating over ten days ($p \leq 0.01$). Furthermore, edible antimicrobial coatings have been related to increase in proton concentration, thereby decreasing the external pH. The change in pH caused by antimicrobial coatings may affect the integrity and permeability of microbial cell membranes disturbing the nutrient transport system causing cell death (Lucera et al. 2012). Additionally, studies have shown color is a good indicator of pH. Meat with a lighter color, such as the meat with the antimicrobial coating in this experiment, is an indicator of lower pH. The lighter color is believed to be due to low pH causing proteins in the muscle to spread out, causing the light to reflect directly from the surface (Mir et al. 2017).

Effect of Antimicrobial Coating on the Rancidity of Chicken Breasts

Figure 9 shows the effect of Carrageenan/Chitosan coating alone or in combination with 20 or 200 ppm of AITC on the rancidity of chicken breasts. The rancidity was measured via a TBARS test, and the results were reported as Malondialdehyde (MDA µg/mL) at 532 nm. Measuring MDA is a method widely used to detect rancidity because MDA are due to second stage auto-oxidation and represent compounds that are responsible for off-flavors or odors produced during storage (Kang et al. 2013). The initial MDA concentration of the chicken breast without coating (positive control) was 0.11. Over the 21-day storage period, the control's MDA concentration increased to 0.72. The initial concentration of MDA in samples with coating, coating + 20 ppm AITC and coating + 200 ppm AITC were 0.19, 0.56, and 0.22. During the 21 days of

storage the MDA concentrations change to 0.76, 1.08, and 0.94. The MDA concentration for all experimental samples was significantly higher than in the control over 21 days ($p < 0.05$).

Similar to the results of this experiment, Azimzadeh et al. (2018) reported that the TBARS values increased steadily over storage periods. However, Azimzadeh et al. (2018) also reported that adding a 1% Chitosan coating with *L.nobilis* extract resulted in significantly lower TBAR values on coated cashews when compared to the control over a 90-day storage period. Bourtoom et al. (2008) reported that a chitosan coating might also decrease TBARS because it may protect the product from exposure to oxygen. Another study also found that adding 1000 ppm of AITC to chicken samples and storing them for up to six days significantly decreased TBAR values ($p < 0.05$) (Hussein et al. 2019). The reasons why the results of this TBAR experiment differ from previous research are not known. However, it is possible that Carrageenan/Chitosan or AITC are causing byproducts similar to MDA that alter the TBAR test because samples with coating, coating + 20 and coating + 200 ppm of AITC did not display the key sensory changes expected from rancidity.

In conclusion a Carrageenan/Chitosan coating with Allyl Isothiocyanate (AITC) increased the shelf life of the product however, they were significant changes in color and rancidity that may indicate a decrease in quality.

LIST OF TABLES AND FIGURES

Table 1

	Lightness				Red/Green				Blue/Yellow			
Day	Control	Coating	Coating + 20ppm	Coating + 200 ppm	Control	Coating	Coating + 20ppm	Coating + 200 ppm	Control	Coating	Coating + 20ppm	Coating + 200 ppm
0	62.4 ± 2.66^{Aa}	64.5 ± 1.62^{Aa}	63.8 ± 4.71^{Aa}	68.2 ± 2.6^{Aa}	0.8 ± 0.89^{Aa}	0.8 ± 2.44^{Aa}	0.7 ± 0.68^{Aa}	1.4 ± 1.0^{Aa}	7.9 ± 1.78^{Aa}	7.2 ± 2.77^{Aa}	7.0 ± 1.96^{Aa}	8.0 ± 4.4^{Aa}
3	59.1 ± 8.11^{Aa}	63.4 ± 1.35^{ABba}	64.6 ± 2.32^{ABa}	69.3 ± 0.8^{Ba}	2.5 ± 0.87^{Aa}	1.7 ± 1.15^{ABa}	0.5 ± 0.4^{BCa}	0.1 ± 0.3^{Cb}	6.1 ± 0.99^{Aab}	7.3 ± 0.28^{Aa}	7.4 ± 3.56^{Aa}	9.0 ± 1.9^{Aa}
6	59.2 ± 4.19^{Aa}	64.5 ± 3.06^{ABab}	62.7 ± 3.54^{ABa}	67.9 ± 1.8^{Ba}	1.8 ± 0.73^{Aa}	0.7 ± 1.96^{Aa}	0.2 ± 0.6^{Aa}	0.1 ± 1.2^{Aab}	4.7 ± 1.91^{Aab}	5.2 ± 0.77^{Aa}	6.5 ± 1.97^{ABa}	8.9 ± 1.8^{Ba}
9	57.8 ± 3.72^{Aa}	61.6 ± 4.81^{ABabc}	65.1 ± 2.95^{BCa}	68.9 ± 3.3^{Ca}	2.2 ± 2.35^{Aa}	1.2 ± 1.47^{Aa}	1.5 ± 0.81^{Aa}	0.5 ± 0.8^{Ab}	5.1 ± 3.37^{Aab}	4.7 ± 0.97^{Aa}	8.2 ± 3.01^{Aa}	9.6 ± 3.2^{Aa}
12	56 ± 4.01^{Aa}	62.5 ± 0.46^{Bbc}	66.2 ± 2.26^{BCa}	67.9 ± 1.2^{Ca}	1.3 ± 0.94^{Aa}	1.1 ± 1.01^{Aa}	0.2 ± 2.07^{Aa}	0.8 ± 1.4^{Aab}	4.2 ± 2.43^{Aab}	6.6 ± 3.61^{ABa}	7.7 ± 2.87^{ABa}	9.2 ± 2.7^{Ba}
15	58.1 ± 4.35^{Aa}	59.6 ± 3.03^{ABbcd}	61.1 ± 7.76^{ABa}	68.3 ± 2.3^{Ba}	0.9 ± 0.43^{Aa}	0.7 ± 0.52^{Aa}	0.7 ± 0.59^{Aa}	0.2 ± 0^{Bb}	5.6 ± 3.56^{Aab}	5.1 ± 3.79^{Aa}	6.4 ± 0.8^{Aa}	8.7 ± 1^{Aa}
18	56.5 ± 4.55^{Aa}	58 ± 3.01^{Acd}	64.8 ± 0.91^{Ba}	67 ± 2.8^{Ba}	2.3 ± 0.51^{Aa}	1.9 ± 1.34^{Aa}	1 ± 1.18^{BAa}	0.1 ± 0.1^{Bb}	4.9 ± 2.11^{Aab}	3.2 ± 0.4^{Ab}	9.9 ± 2.53^{Ba}	8.3 ± 1.1^{Ba}
21	55.7 ± 3.61^{Aa}	55.9 ± 3.00^{Ad}	64.9 ± 4.87^{Ba}	69.8 ± 1.8^{Ba}	2.3 ± 0.47^{Aa}	1.9 ± 0.49^{Aa}	1.2 ± 1.13^{ABa}	0.1 ± 0.4^{Bb}	3.3 ± 1.03^{Ab}	4.8 ± 3.32^{Aa}	10.4 ± 2.92^{Ba}	10.7 ± 3.2^{Ba}

Figure 1

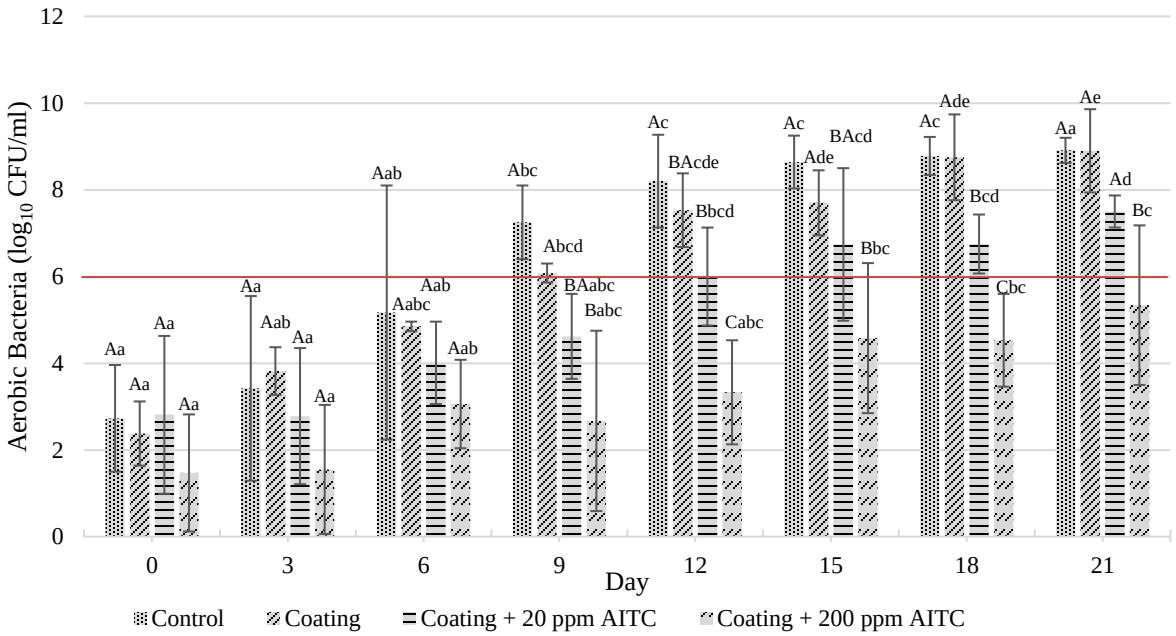

12
10
8
6
4
2
0
Aerobic Bacteria (log10 CFU/ml)
Aa
Aa
Aa
Aa
Aa
Aab
Aa
Aab
Aa
Aab
Aabc
Aab
Abc
Abcd
BAabc
Babc
Ac
BAcde
Bbcd
Cabc
Ac
Ade
BAcd
Bbc
Ac
Ade
Bcd
Cbc
Aa
Ae
Ad
Bc
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + 20 ppm AITC
Coating + 200 ppm AITC

Figure 2

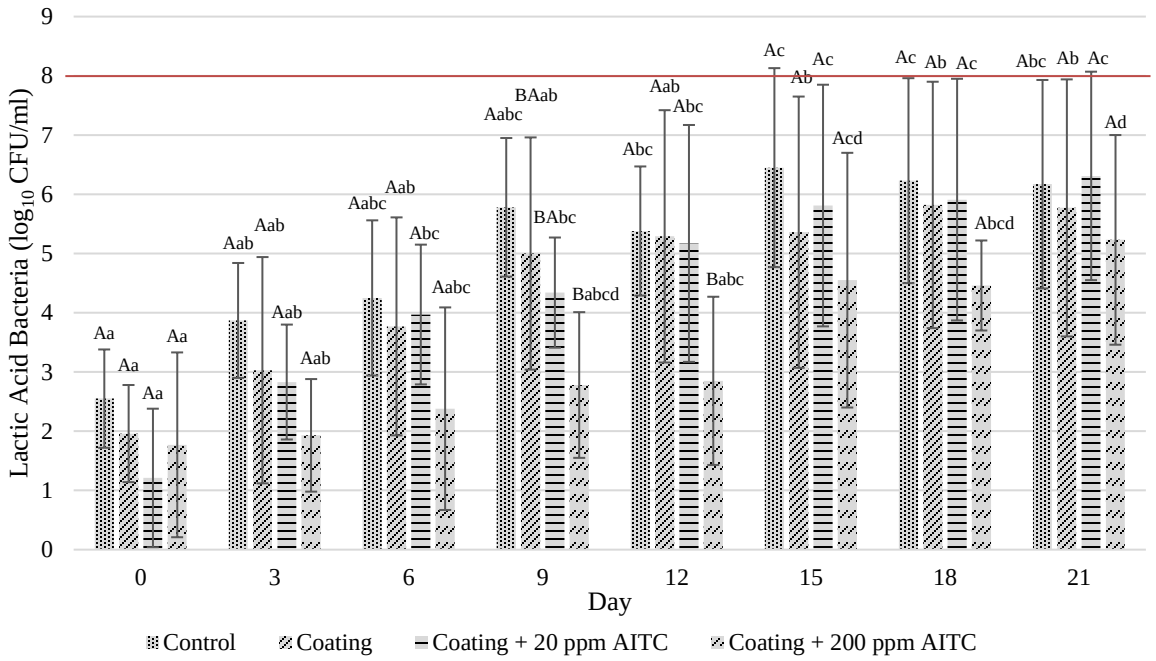

Lactic Acid Bacteria (log10 CFU/ml)
9
8
7
6
5
4
3
2
1
0
Aa
Aa
Aa
Aa
Aab
Aab
Aab
Aab
Aabc
Aab
Abc
Aabc
Aabc
BAab
BAbc
Babcd
Abc
Aab
Abc
Babc
Ac
Ab
Ac
Acd
Ac
Ab
Ac
Abcd
Abc
Ab
Ac
Ad
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + 20 ppm AITC
Coating + 200 ppm AITC

Figure 3

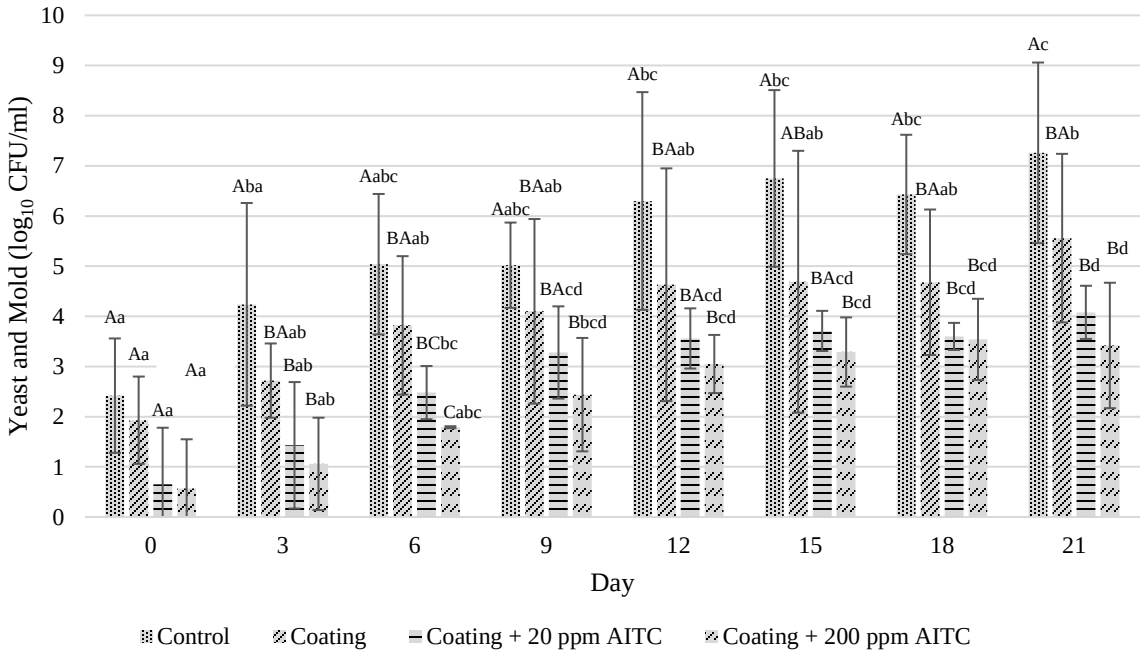

Yeast and Mold (log10 CFU/ml)
10
9
8
7
6
5
4
3
2
1
0
Aa
Aa
Aa
Aa
Aba
BAab
Bab
Bab
Aabc
BAab
BCbc
Cabc
Aabc
BAab
BAcd
Bbcd
Abc
BAab
BAcd
Bcd
Abc
ABab
BAcd
Bcd
Abc
BAab
Bcd
Bcd
Ac
BAb
Bd
Bd
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + 20 ppm AITC
Coating + 200 ppm AITC

Figure 4

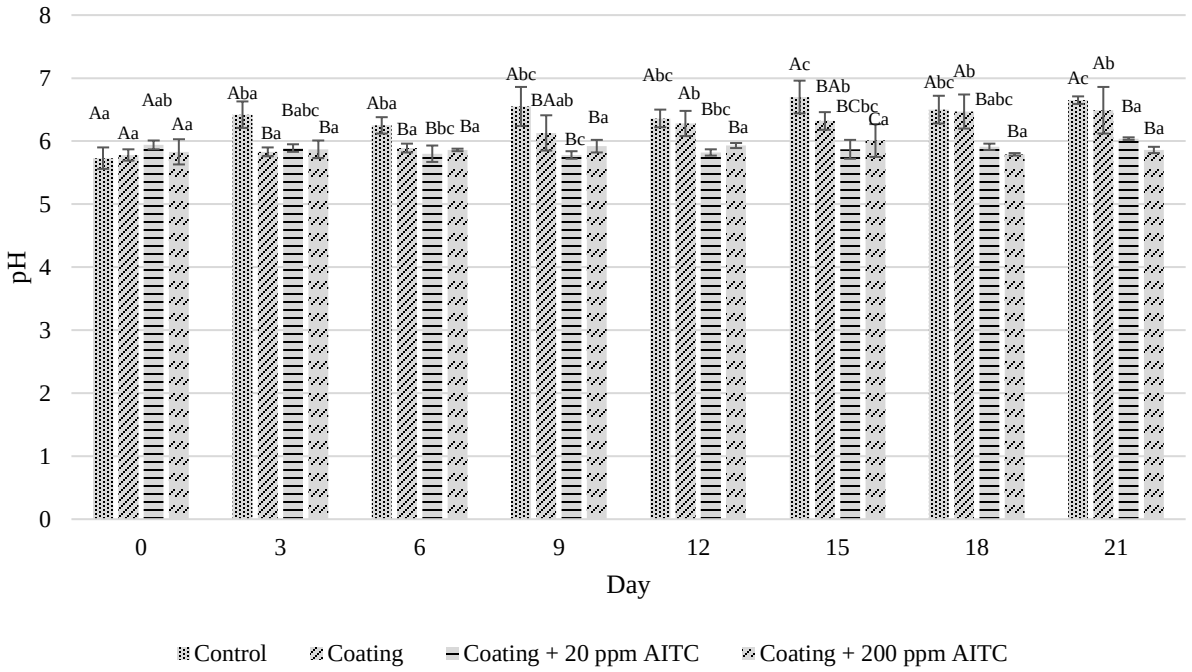
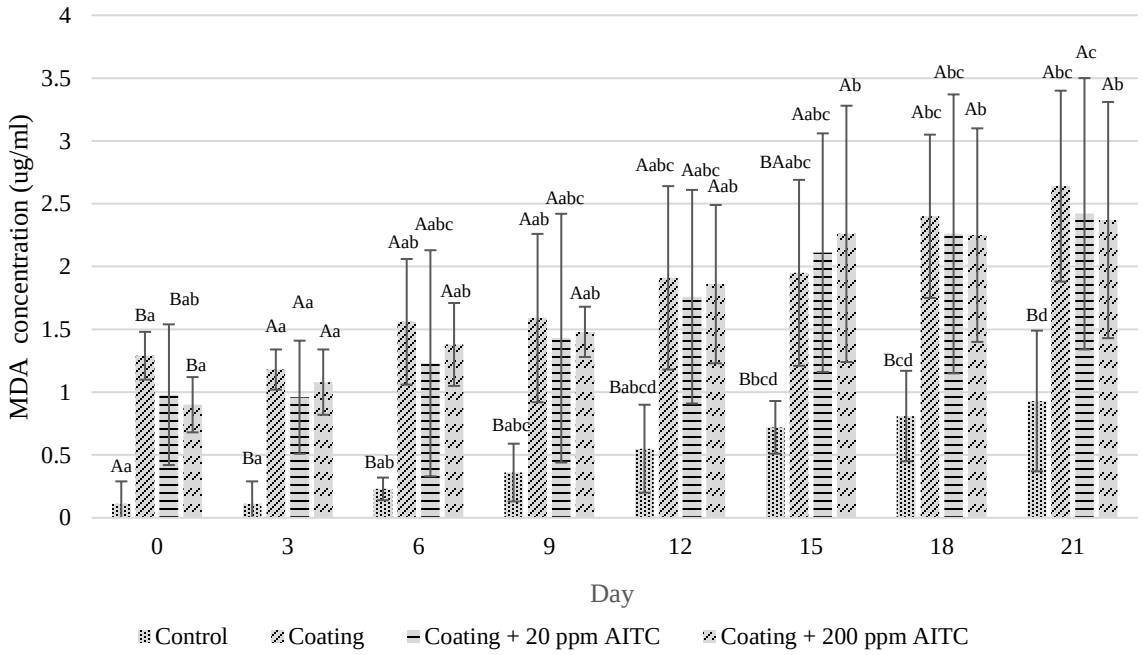

8
7
6
5
4
3
2
1
0
pH
Aa
Aa
Aab
Aa
Aba
Ba
Babc
Ba
Aba
Ba
Bbc
Ba
Abc
BAab
Bc
Ba
Abc
Ab
Bbc
Ba
Ac
BAb
BCbc
Ca
Abc
Ab
Babc
Ba
Ac
Ab
Ba
Ba
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + 20 ppm AITC
Coating + 200 ppm AITC

Figure 5


MDA concentration (ug/ml)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
3
6
9
12
15
18
21
Day
Control
Coating
Coating + 20 ppm AITC
Coating + 200 ppm AITC
Aa
Ba
Bab
Ba
Ba
Aa
Aa
Aa
Bab
Aab
Aabc
Aab
Babc
Aab
Aabc
Aab
Babcd
Aabc
Aabc
Aab
Bbcd
BAabc
Aabc
Ab
Bcd
Abc
Abc
Ab
Bd
Abc
Ac
Ab

REFERENCES

Azimzadeh, B., & Jahadi, M. (2018). Effect of chitosan edible coating with *Laurus nobilis* extract on shelf life of cashews. *Food Science and Nutrition, 6,* 871-877. https://dx.doi.org/10.1002%2Ffsn3.630

Barbut, S. (1993). Colour measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. *Food Research International,* 26, 39-43. https://doi.org/10.1016/0963-9969(93)90103-P

Bourtoom, T. (2008). Edible films and coatings: Characteristics and properties. *International Food Research Journal, 15,* 237-248. https://doi.org/10.5937/FFR1501011S

Buzby, J. C., Wells, H. F., Axtman, B. & Mickey, J. (2009). Supermarket loss estimates for fresh fruit, vegetables, meat, poultry, and sea food and their use in the ERS loss-adjusted food availability data, *United States Department of Agriculture, Economic Research Service, Economic Information Billeting*.

Gill, C. O. (2003). Active packaging in practice: meat. *Novel Food Packing Techniques. 1,* 365-383. https://doi.org/10.1533/9781855737020.3.365

Graumann, G. H., & Holley, R. A., (2009). Survival of *E. coli* O157:H7 during manufacture if dry-cured Westphalin ham surface-treated with allyl isothiocyanate or hot mustard powder. *Journal Science Food Microbiology, 89,* 617-624. https://doi.org/10.1002/jsfa.3487

Hussein, K., Friedrich, L., Kisko, G., Ayari, E., Nemeth, C., & Dalmadi, I. (2019). Use of allyl-isothiocyanate and carvacrol to preserve fresh chicken meat during chilling storage. *Czech Journal of Food Science, 37,* 417-424. https://doi.org/10.17221/80/2019-CJFS

Kang, H. J., Kim, S. J., & You, Y. S. (2013). Inhibitory effect of soy protein coating formulations on walnut kernels against lipid oxidation. *LWT-Food Science and Technology, 51*, 393-396. https://doi.org/10.1016/j.lwt.2012.10.019

Kim, S. A., Park, S. H., Lee, S. I., Owens, C. M., & Ricke, S. C. (2017). Assessment of chicken carcasses microbiome responses during processing in the presence of commercial antimicrobials using a next generation sequencing approach. *Scientific Reports*, *7*, 433-454. https://doi.org/10.1038/srep43354

Kim, C. R. (2000). Quality evaluation of refrigerated chicken wings treated with organic acids. *Journal of Food Quality*, 23, 327-335. https://doi.org/10.1111/j.1745-4557.2000.tb00218.x

Ko, J. A., Kim, W. Y., & Park, H. J. (2011). Effect of microencapsulated Allyl isothiocyanate (AITC) on the extension of shelf-life of Kimchi. *International Journal of Food Microbiology, 153*, 1-2. https://doi.org/10.1016/j.ijfoodmicro.2011.10.021

Lucera, A., Costa, C., Conte, A., & Del Nobile, M. A. (2012). Food applications of natural antimicrobial compounds. *Frontiers in Microbiology, 3*, 287. https://doi.org/10.3389/fmicb.2012.00287

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shuckla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of Food Science and Technology, 54*, 2997-3009. https://dx.doi.org/10.1007%2Fs13197-017-2789-z

Olaimat, A. N., & Holley, R. A. (2014). Control of *Salmonella* on fresh chicken breasts by k-carrageenan/chitosan based coatings containing allyl isothiocyanate or deodorized Oriental mustard extract plus EDTA. *Food Microbiology.* https://doi.org/10.1016/j.fm.2014.11.019

Redondo-Blanco, S., Fernandez, J., Lopez-Ibanez, S., Miguelez, E. M., Vilalr, C. J., & Lombo, F. Plant phytochemicals in food preservation: Antifungal bioactivity: A review. *Journal of Food Protection, 83*, 163-171.

Remington H. (2017). Achieving sustainable production of poultry meat, Volume 1: safety, quality and sustainability. *British Poultry Science*, *58*, 469. https://doi.org/10.1080/00071668.2017.1355511

United States Department of Agriculture. (2016). Top 50 Ranking of Chicken Cuts. *USDA*

United States Department of Agriculture. (2020) Broiler Chicken Industry Key Facts 2020. *National Chicken Council.*

Wagle, B. R., Upadhyay, A., Shreshtha, S., Arsi, K., Upadhyaya, I., Donoghue, A. M., & Donoghue, D. J. (2019). Pectin or chitosan coating fortified with eugenol reduces *Campylobacter jejuni* on chicken wingettes and modulated expression of critical survival genes. *Poultry Science, 98*, 1461-1471. https://doi.org/10.3382/ps/pey505

Winther, M., & Nielsen, P. W. (2006). Active packaging of cheese with allyl isothiocyanate, an alternative to modified atmosphere packaging. *Journal of Food Protection, 69*, 2430-2435. https://doi.org/10.4315/0362-028x-69.10.2430

Wu, Shengjun. (2014). Effect of chitosan-based edible coating on preservation of white shrimp during partially frozen storage. *International Journal of Biological Macromolecules, 65*, 325-328. https://doi.org/10.1016/j.ijbiomac.2014.01.056

Xia, W., Liu, P., Zhang, J., & Chen, J. (2011). Biological activities of chitosan and chitooligasaccharides. *Food Hydrocolloids. 25*, 170-179. http://dx.doi.org/10.1016/j.foodhyd.2010.03.003

Yegappan, R., Selvaprithiviraj, V., Amirthalingam, S., & Jayakumar, R. (2018). Carrageenan based hydrogels for drug delivery, tissue engineering and wound healing. *Carbohydrate Polymers, 198,* 385-400. https://doi.org/10.1016/j.carbpol.2018.06.086