**Shelf Life Validation of Marinated and Frozen Chicken Tenderloins**

by

John Gregory Rehm

A thesis submitted to the Graduate Faculty of
Auburn University
In partial fulfillment of the
Requirements for the Degree of
Master of Science

Auburn, Alabama
August 8, 2020

Approved by

Dr. Christy Bratcher, Chair, Professor of Animal Science
Dr. Jason Sawyer, Associate Professor of Animal Science
Dr. Amit Morey, Assistant Professor of Poultry Science

## Abstract

It is immensely important for producers and restaurants to know the shelf life of a meat product. If a consumer eats a product that is rancid it could impact a restaurant's reputation. The objective of this study is to validate the shelf life of marinated and frozen chicken tenders. The treatments were the age of the chicken tender after harvest, which were 4 days of age (DA), DA5, DA6, DA7 and DA8. Spoilage organisms, pH and instrumental color (L*, a*, b*) were measured to assess the shelf life of bulk-packaged bags of chicken tenders. The microbial analysis analyzed the growth of aerobic, psychotrophic and lactobacilli bacteria. Each treatment contained 47.63 kg of chicken. Chicken was sampled fresh then tumbled in a marinade that contained water, salt, modified corn starch and monosodium glutamate. After marinating, the chicken tenders were sampled (0 hours) and the other remaining tenders were put into a blast freezer (-25ºC). After freezing, the chicken thawed in a cooler (2.2ºC) for 132 hours (h) and was sampled at 36h, 60h, 84h, 108h, 132h. After marinating the chicken tenders, each treatment decreased in the aerobic count and the psychotroph count except for DA4. During the thawing test no treatment crossed the microbial threshold of $10_6$ CFU/mL. Although none crossed the threshold for this study, treatments DA4, DA5, DA6 had a spike ($P < 0.05$) in aerobic bacteria when the treatment would have been technically 7 days of age. The psychotrophic bacteria continuously grew at each thaw sampling period with the earlier treatments making larger increases in growth. Both DA4 and DA5 surpassed the other treatments ($P < 0.05$) at 108h and 132 h reaching $10_5$ CFU/mL. Every treatment remained below the microbial threshold, which means that they are not spoiled on a microbial basis.

**Acknowledgements**

I want to thank my Lord Jesus Christ, for this incredible journey and opportunity to meet such amazingly talented people. The countless memories made throughout this two-year program will never be forgotten. Thank you to my parents and siblings for always being supportive the entire time of the program.

I am greatly appreciative for the Department of Animal Sciences and Poultry Sciences. The faculty's knowledge and comradery helped me to grow into who I am today. I want to thank Dr. Sawyer for keeping me on track during my last year at Auburn University. I am grateful for Dr. Morey, and Dr. Garner, the lab training and exposure allowed for me to pursue my research. I am very thankful for Dr. Childs and Katie Sowell; you both were very patient with me and always so generous. To the graduate students, the memories made were unforgettable. You all helped keep me my head up throughout the years. Shannon Wilkerson, Alex Tigue, Alisha Kerklin, and Hunter Smith; you all were always fun to work with and an amazing team.

To Dr. Bratcher, words cannot explain how thankful I am for this opportunity. Thank you for allowing me to join this amazing team at Auburn University and for being a great mentor from the beginning of my meat science career. Thank you Barney Wilborn, for mentoring me into a young professional and for being patient with me at the meat lab. You both have made such an enormous impact on my life and I hope that I have made both of you proud. I cannot wait to meet again and talk about the good ole days. I hope to stay in touch with this amazing team and hope to see you all again in the future.

## Table of Contents

## List of Tables

## List of Figures

# Chapter I Literature Review

## Poultry Industry

The poultry industry is continuously growing in both production and efficiency. Meat originating from the carcass of any domesticated bird is considered a poultry product, as defined by the Poultry Products Inspection Act (FSIS, 2016). In the early 1900's the market weight for a chicken was 1.13 kg and now producers have the capability to produce a bird that weighs over 2.27 kg in 47 days (Barbut et al., 2015). With the advancements in technology, genetics and production the poultry industry has allowed for more specialization and variety to provide customers. One of the most popularly consumed proteins in the United Sates is poultry. In 2018 there was 25.7 million kg of chicken produced in the United States of America according to the United States Department of Agriculture/ National Agricultural Statistics Service (USDA/NASS). The USDA/ NASS Poultry Slaughter 2018 summary states that the poultry industry valued broilers at 31.7 billion dollars, which was a 5% increase from the previous year in 2017. The demand for poultry is increasing, in 2014, per capita consumption was 38.01 kg and 5 year later, increased to 43.64 kg with an expected 4.5% increase in 2020 according to the National Chicken Council, per capita of poultry and livestock, 1960 to forecast 2020 report. Poultry is not expensive in comparison to other proteins, it has a short growth period and most religions allow consumption of poultry (Barbut et al., 2015). Each year chicken producers are becoming more efficient and producing a larger volume to meet the global demand. Chicken products can be found at grocery stores, fast food chains and restaurants. This creates a need for diversity in the type of processing plants.

## Poultry Harvest

For chicken to be on the plate of a consumer it undergoes a multitude of processing steps. There are large poultry processing plants that have the capabilities to process a bird from harvest to a consumer product that can be shipped to a retail store or foodservice entity. These types of processing plants typically have a harvesting floor adjacent to a de-boning (portioning) floor to eliminate the shipment/logistical movement of chicken parts and pieces from one processing facility to another. The following flow chart outlines the typical harvesting process of chicken.

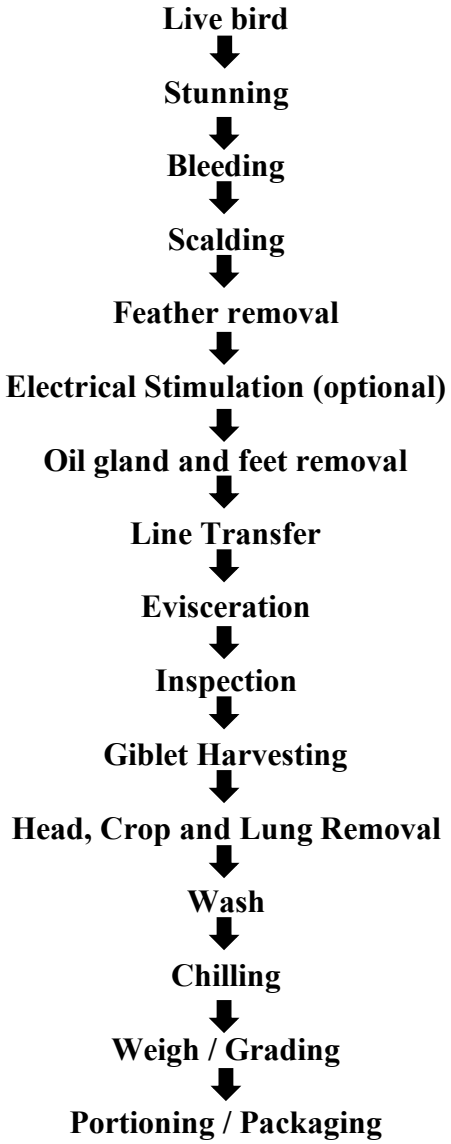


Figure 1. This flow chart was adapted from Barbut et al., 2015.

Some facilities are dedicated solely to harvesting a specific type of bird (chicken, turkey, duck etc.) depending upon growers in the region of the processing facility. Facilities in the past were only capable of processing one to two thousand birds per hour, but with advancements in technology, plants can now process as many as 13,000 birds per hour (Barbut et al., 2015). Automation has changed the industry and allowed for the producer to meet this large increase in demand of poultry. This increase in poultry has also increased the demand for more specific types of products. Not all processing plants have the capability of creating every item that is available for purchase in grocery stores and thus portioning plants were developed.

**Portioning**

Within the meat industry there are plants that are dedicated to portioning meat into specific items. These items may go directly into the foodservice sector or possibly another plant, it all depends on the type of product that is being produced. The main reason for these pre-portioned products is because it is more convenient for the consumer (Barbut et al., 2015). According to Barbut 2015, whole birds made up 85% of the market but in 2013 that number of whole birds had decreased below 10%. Typically, a portioning facility will receive either front quarter, (wings, wing drumettes, breast, tender and breast carcass) or the hind quarter (thigh meat and drumstick) to be further processed into additional consumer pieces. The specific type of meat that the plant will receive depends on the production schedule of the plant and the capabilities of production. One product of particular popularity is chicken tenders. The chicken tender is a product that comes from the *supracoracoideus*, which is located below the *pectoralis* muscle or commonly referred to as the breast (Barbut et al., 2015). The common name for the *supracoracoideus* is the tender. The chicken tender is often found at grocery stores in an expanded polystyrene tray with poly-vinyl chloride film or at a restaurant served as a breaded or

grilled product. For the tender to reach its final destination it will go through multiple processing steps following harvest and fabrication

The chicken tender is a common product that is produced in these portioning plants. Foodservice poultry customers typically desire a chicken tender that is uniform size. This can be a tough goal to accomplish because tenders are quite delicate and can be damaged easily during de-boning. In order for the processor to meet the expectation of some foodservice customers, they must split the tenders so that they are within the target weight and length ranges. For the processor to meet the weight and length target the processor will either use hand trimmers or mechanically trim the chicken tenders. This process can be very harsh on the tenders and cause them to become damaged.

Cutting the tenders makes them even more delicate than they already were because the membrane that surrounds the muscle is torn. After portioning the product, the processor may tumble the tenders in a solution marinade. Typically, this is accomplished via a vacuum tumbler. The purpose for vacuum tumbling is aid in distributing the marinade throughout the protein and for improving protein extraction (Barbut et al., 2015). Typically, a poultry marinade contains water, salt, and phosphate. In addition to adding moisture and flavor, another major contribution from salt within a marinade solution is extraction of the salt soluble proteins during the tumble massaging process, (Barbut et al., 2015) of the actin and myosin. The use of phosphate can improve water-holding capacity (WHC) of the chicken tender by increasing or decreasing postmortem muscle pH of the product (Barbut et al., 2015).

**Muscle Structure**

Meat comes from an animal and is made up of multiple cells and structures. The type of muscle tissue discussed in this paper is skeletal muscle. Skeletal muscle is anchored by tendons that move and support the animal. Skeletal muscle has a striated appearance when viewed through a microscope because skeletal muscle contains sarcomeres with light and dark bands. The sarcomere is the smallest contractile unit of a muscle and contains two major proteins which are myosin (thick filaments) and actin (thin filaments). These two proteins work together to create muscle contraction and relaxation. Each sarcomere is held together by the Z-line. Sarcomeres link together to create muscle fibers, which are surrounded by a layer of connective tissue known as the endomysium (Barbut et al., 2015). The muscle fibers are bundled together and referred to as a muscle bundle, which is wrapped in another layer of connective tissue known as the perimysium. The outer most layer of connective tissue is the epimysium and it surrounds the entire muscle which is comprised of countless muscle bundles.

Muscle fibers are classified based on their fiber type which can be either red or white. The red muscle fibers have been associated with slow contractile speeds and are capable of conducting muscle contraction for longer periods of time. Muscles that contain mostly white muscle fibers are found in areas of the chicken that are used for short bursts of energy such as the breasts (Barbut et al., 2015) and can be linked to faster contractile speeds.

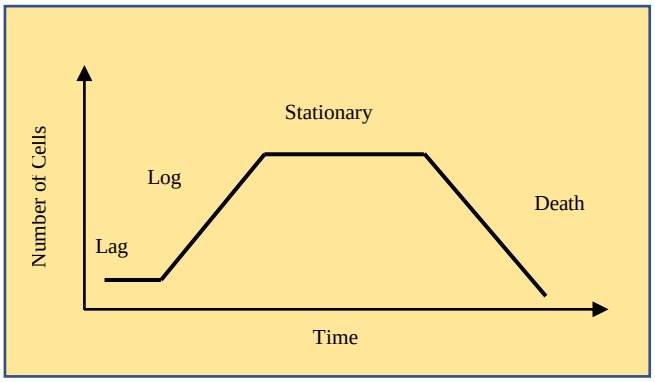
## Spoilage and Waste

### What is spoilage?

Consumers can recognize spoilage of a meat product when the meat begins to physically deteriorate and produce strong odors. Spoilage can be caused by both extrinsic and intrinsic factors. Temperature, relative humidity and oxygen availability are extrinsic factors. Most

microorganisms have a specific temperature range that is favorable for growth and multiplication. Thermophiles are microorganisms that prefer temperatures exceeding 45°C, while psychrophiles grow at temperatures less than 20°C. The microorganisms that grow in the range between thermophiles and psychrophiles are mesophiles (Judge et. al, 1989) at a temperature range of 20ºC to 45ºC. The life cycle of microorganisms occurs in four phases, which can be explained as the lag phase, the log or exponential (growth) phase, the stationary phase and the death phase (Doyle et al., 2001).

Figure 2. Microorganism Growth Phases




In the lag phase the microbial cell/microorganisms are becoming situated in its' new environment, an environment that provides nutrients (food, water, oxygen) for growth. The log phase is the time in which the microbial cell begins to divide into two cells, then four and multiplying rapidly (Doyle et al., 2001) and if allowed will increase their total population. These two phases are very important for the growth of the microorganism. Following the log phase is the stationary phase where microbial growth begins to plateau (slow) with replication of microbial cells become limiting due to lack of nutrients and will eventually decline during the death phase. Altering the environmental conditions will affect the stage of microbes along this growth curve.

**Extrinsic Factors**

Since microorganisms have varying growth and survival temperatures, changing the temperature of the environment can inhibit that specific microbe's growth rate. Altering the storage temperature of a food or meat product outside of the optimal microbial growth and survival range does not completely eliminate growth of a microorganism, rather it can impede the growth rate of microorganisms. As the storage temperature increases so does the rate of growth and it is plausible that with every increase in 10°C of storage temperature the growth rate doubles (Doyle et al., 2001).

Relative humidity can be responsible for sweating, which is when moisture condenses onto the meat typically at humidity above 88 to 92 percent (Judge et. al, 1989). Moisture on the surface of the meat can lead to microbial growth because microorganisms have a requirement for water (Judge et. al, 1989). Microorganisms require varying levels of oxygen, moisture and nutrients for maintaining the basic processes of life. Aerobic bacteria need oxygen, while anaerobic bacteria can survive without free oxygen. Another classification of bacteria referred to as facultative organisms, can live with or without oxygen. While extrinsic factor of microorganism survival is the meat or food product itself. Meat products that have been cut, chopped or ground have a greater likelihood of microorganism contamination. Therefore, meat products tend to be more susceptible to spoilage through microbial activity (extrinsic) and the biochemical reactions (intrinsic) of the individual meat components, specifically protein, fat and water (Doyle et al., 2001).

**Intrinsic Factors**

Intrinsic factors include water activity, pH level of the meat, oxidation-reduction, nutrients and tissues such as fat. Microorganisms require water for growth and survival within a food or meat product matrix. As available water within the meat product declines, less water is available for microorganisms. Water activity is used to express the amount of water a microorganism needs for survival. Doyle et al., (2009) states that bacteria need a greater-water activity for survival with the minimum hoovering near 0.81 to 0.90 $a_w$. Therefore, meat can be a suitable substrate for bacterial growth due to its available water.

Microorganisms also need nutrients other than water in order to grow. Meat is comprised of protein, allowing the microorganisms to obtain nitrogen through consumption of protein for growth and survival. Preventative growth of other microorganisms, through competitive inhibition, allows for one microorganism species to grow and use the available nutrients while inhibiting the survival of other microorganism species.

A neutral pH near 7.0 is ideal for most microorganism growth and survival. Commonly, the pH of a poultry carcass will range from 5.8 to 6.0 after postmortem pH declines following harvest and chilling. Depending on the classification of the microorganisms, either an oxidized state or a reduced state is preferential for growth and survival. Aerobic microorganisms typically prefer a greater oxidation-reduction potential, whereas anaerobic microorganisms prefer less oxygen (reducing) indicative of a weaker oxidation-reduction potential. These organisms compete for growth and so the organisms can decrease or increase the oxidation-reduction potential to favor the necessity of that particular microorganism (Judge et. al, 1989).
The surface of meat is the most susceptible location for bacteria to survive and, it is where the oxidation-reduction potential is the greatest (Judge et. al, 1989).

## Economic Implications on Food Waste

The USDA Economic Research Service (ERS) considers food waste to be in the category of food loss, which is edible food that humans are capable of eating but it is not eaten for a multitude of reasons (Buzby et al., 2014) such as spoilage, cooking losses or left on a plate or pan. In a study published by Buzby et al. (2014), the authors found that 31% of the available food supply was not consumed in 2010. The consumer goods made up 21% of the 31% of food loss and this is due to the consumers desire for more specific items (Buzby et al. 2014) such as more wholesome and fresher food products. In the meat industry, if a manufactured meat product does not fit the product specification, then it is repurposed or used in creating a different meat product. However, in the foodservice and retail arenas meat products tend to be discarded if not sold or consumed prior to the specified manufacturer shelf life. Consumers on the other hand will throw out a product due to spoilage because it became unfit for their standards of consumption. According to the Environmental Protection Agency (EPA, 2019) in 2017, food waste accumulated approximately 37.1 million metric tons and that food is the most common material found in trash. Occasionally, a consumer may purchase more than they need, and the excess food will be wasted. Finally, consumers will discard food products when it has reached or passed the manufacturers identified shelf life as indicated by the “Best-By”, “Use-by” or “Freeze-by” date on the package of food.

## Labeling

In the current market settings of foodservice and retail manufacturing, there are multiple terms that are required by USDA Food Safety Inspection Service (USDA-FSIS) on a meat product label. Some food and meat labeling can be confusing to consumers because the industry

terminology used across protein sectors are similar but can define different applications for industry or governmental agencies. Consumers are becoming more knowledgeable of meat product labels as packaging label usage of phrases such as; “natural”, “no antibiotics added”, or “organic”. These phrases refer to how the chicken was raised, or how the product was processed. Whereas, commonly used phrases as it is related to food spoilage are “Best By”, “Sell By”, “Use By”, and “Freeze By”. These phrases provide a tool for the foodservice or retail sector to identify when the meat passes the wholesomeness for consumption. These phrases have been defined by the USDA and made available through a multitude of platforms to educate consumer, customers, and commodity groups.

Identifying meat packages with a “Best if used By” term can describe when the product is at its most optimum best quality and it is not a food safety date. Creating a “Sell-By” date is often for the retailer so that they know when and how long to put a product out for sale and is not a safety date. When describing the date that the product will be at peak quality, the term “Use-By” is considered and placed onto the meat package or box label. The last term often used within the meat industry is “Freeze-By”, which indicates to the foodservice or retail arena when the product needs to be frozen so that it may retain its peak quality and is not a food safety timeline.

These phrases are linked to a Julian calendar day that defines the day of manufacturing for the specific product. Julian day of production is placed onto the product label and can be used by the retail or foodservice customer to make produce wholesomeness decisions. Spoilage bacteria are not considered to cause illness but in the eyes of the consumer (Blackburn et al., 2006), spoilage and food safety go hand in hand. Through competition, some spoilage bacteria can actually prevent pathogen growth (Blackburn et al., 2006), but organisms can also promote growth, which is known as metabiosis. The use of product manufacturing dates (Julian Calendar)

are not required by federal law and are used as an indicator of product quality and not as a measure of food safety.

## Types of Spoilage

### Chemical Spoilage

Hydrolytic enzymes present in meat are capable of metabolizing proteins, lipids and carbohydrates in addition to the degradation of meat by microorganisms. The microorganisms break down these compounds and use them for nutrients. Microorganisms use lipases to hydrolyze triglycerides into glycerol and fatty acids (Judge et al., 1989), while phospholipids are turned into nitrogenous bases and phosphorus.

The breakdown of carbonyl compounds, hydrocarbons and furans via lipid oxidation can cause rancidity and off flavors in meat (Ladikos and Lougovois, 1990) as measured using sensory taste panels. Oxidative rancidity occurs when unsaturated fatty acids react with oxygen. The rancid flavors and odors originate in the form of compounds such as aldehydes and short-chain fatty acids (Pothakos et al., 2015) and can be measured through chemical and physical analysis. Oxidation of meat products can occur in aerobic environments and the rate of oxidation can be dependent upon oxygen quantity and the quantity of unsaturated fatty acids in the fat of the meat or food product. Ready-to-eat meat products can also encounter deterioration as a result of lipid oxidation. When meat is cooked it increases the lipid oxidation rate (Ahn et al., 1992) due to phospholipid structure deterioration. Meat proteins contain metals, such as copper, iron and zinc, which are often classified as free metal ions in a meat structure. These free metal ions can catalyze the oxidation of oxymyoglobin (Kanner, 1994) which is oxidized protein form of myoglobin visible to consumers. The surface color of meat can be oxidized through the process

of cutting, grinding and mechanically separating, because the destruction of muscle membranes increases free radicals through the association of iron with oxygen (Ki-Chang et al., 2002) causing greater quantities of myoglobin to be exposed to oxygen.

**Physical Spoilage**

Physical spoilage identifies the smell, texture, taste and color associated with sensory characteristics of a fresh or cooked meat product. Under vacuum-packaging conditions, the only microorganisms capable of growing under these packaging methods are anaerobic and facultative microorganisms due to the lack of available oxygen in the package. The common physical changes observed in vacuum packaged meat are souring, taint and putrefaction (Judge et al., 1989) that can be detected through sensory analysis. Souring is the development of sour flavors or off odors due to the buildup of organic acids, while taint is a term used to describe off odors and flavors (Judge et al., 1989) when measured using qualitative or quantitative analysis.

**Microbial Spoilage**

Microflora can be found on the surface and within food and meat products that can be found at both the foodservice and retail sectors. These products can be purchased, and plausible spoilage can be detected by rancid odors and visual detection of surface sliminess. The aerobic, anaerobic and psychotrophic microflora are common reasons for causing a meat protein item to appear spoiled (Lambert et al., 1991) or past its wholesome consumption period. Bacteria can come in contact with meat at the manufacturing facility where it travels along and through various pieces of equipment, tabletops or past an employee. The operations that occur within a processing facility can influence the quantity of bacteria that could end up on the product

(McDonald et al., 1999) surface or within the packaged product. As a poultry product is further processed, the microbial load can increase. For example, a cut-up piece of poultry will have greater total microbial quantities than a whole muscle product (Doyle et al., 2009) as indicated through analytical measures. In 2007, Dominguez et al., conducted a study that examined the impact of temperature on the growth rate of bacteria. The authors (Dominguez et al., 2007) found that small changes in storage temperatures can have an impact on shelf life of a meat product due to the growth of microorganisms occurring faster at warmer storage temperatures. Lastly, it is important to consider all steps of processing a meat product will go through, from harvesting and packaging to logistics and consumption at the foodservice or retail consumer level. There are many opportunities during meat and poultry processing for products to come in contact with bacteria.

**Microbial Testing**

Spoilage can occur from microorganisms growing on the surface and within meat. Microbial growth predictions can be developed through microbial plating (sampling a representative amount of meat and providing the specific nutrients for that organism and temperature on a petri dish) to determine the total viable count (Doyle et al., 2009) of bacteria. There is a variety of plate agars and storage temperatures that can be used in order to test for specific bacteria. The bacteria are plated onto the media and incubated for a specified time. Research suggested (Barbut et al., 2015) that typically slime, and off odor tends to be detected at or near $10_6$ to $10_8$ colony forming units / gram (CFU/g) of the sampled product. Although this method can be used to gather Total Viable Count (TVC) of different species of bacteria (Blackburn et al., 2006) it does not necessarily explain which bacterium is causing the spoilage

metabolites to be produced. Products may not be spoiled due to a larger TVC and therefore it is important to note the sensory qualities of the product. In a test conducted by Desai et al. (2014) the shelf life of fresh chicken breasts placed into a vacuum package with the addition of vinegar were evaluated. In their conclusion (Desai et al., 2014), packages of chicken breast without vinegar solution began to approach a spoilage threshold on day 12 ($10_6$ CFU/g) and deemed spoiled by day 16, due microbial growth ($10_8$ CFU/g) that was reported. This data indicated that microbial and sensory results can determine the shelf life of a meat product. Lastly, it is important to note that spoilage is a subjective term and bacterial counts can vary from day to day (Doyle et al., 2001) throughout the shelf life of a meat product.

## Specific Spoilage Organisms

### ***Pseudomonas spp.***

One of the most common spoilage microorganisms found in the meat and dairy industries is *Pseudomonas. Pseudomonas spp.* is a psychrotrophic bacteria and therefore can grow in the cold refrigerators or coolers that meat products are stored in (Stellato et al., 2017). Stellato et al. (2017) located the two most commonly found species of the *Pseudomonas spp.* being *Pseudomonas fragi* and *Pseudomonas fluorescens.* Glucose, amino acids and lactic acid are substrates that *Pseudomonas spp.* consume under aerobic conditions to replicate (Lambert et al., 1991) as research suggests. Lambert et al. (1991) reported that the end products of *Pseudomonas* growth were surface slime, sulfides, esters, acids and amines. *Pseudomonas* spp. is a Gram-negative bacteria as Doyle et al. (2009) explains that Gram-negative bacteria require greater water activity (0.93 to 0.96) for growth and survival, which is found in most poultry products.

## Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) is a category of bacteria that are Gram-positive, catalase negative, can be classified as either microaerophilic or facultative anaerobes, and are gas fermenters (Doyle et al., 2009). Growth of LAB can lead to specialty food products through fermentation, such as salamis or cheeses, but LAB can also cause create characteristics of spoilage in foods such as fresh meat, luncheon meats and dairy products. These two forms of LAB are capable of fermentation. Homo-fermentative lactics are capable of growing without oxygen, which is known as fermentative metabolism (Doyle et al., 2001). The hetero-fermentative lactics (Doyle et al., 2009) have the capability of producing lactic acid, carbon dioxide and ethanol or even acetic acid when they ferment glucose. These microorganisms need an electron acceptor because there is no oxygen available. Aerobic organisms use the electron transport chain, which requires oxygen as the electron acceptor during oxidative phosphorylation (Doyle et al., 2001) phases. Anaerobic bacteria must use fermentation to generate ATP because the bacteria lack a functional electron transport chain.

In a vacuum package, there is a limited oxygen presence, which creates an environment that is favorable for LAB growth since they are facultative anaerobes (Pothakos et al., 2015). The first nutrient that is consumed is glucose and when it is consumed by the LAB the fermentation does not create a rancid or off odor smell (Pothakos et al., 2015). Although, by metabolizing sugar the bacteria are capable of producing lactic acid, which produce a sour taste (Doyle et al., 2009) that can be measured by sensory analysis. Once glucose is consumed, the microbiota needs additional substrates to consume for continued exponential growth of microorganisms. Other substrates of LAB tend to include; lactate, gluconate, pyruvate, propionate, ethanol, amino acids and nucleotides (Nychas et al., 2008).

***Shewanella putrefaciens***

*Shewanella putrefaciens* is a Gram-negative bacterium commonly found in marine environments. *Shewanella putrefaciens* is a facultative anaerobic bacterium that can also be found in vacuum packaged products and poultry products. The bacterium has been shown (Moser et al., 1996) to grow by turning sulfur from amino acids such as cystine into hydrogen sulfide ($H_2S$) a common gas produced from deterioration of chicken meat products. *Shewanella. putrefaciens* is capable of breaking down these amino acids with the assistance of glucose, which can give the bacteria an advantage for growth (Blackburn et al, 2006) and replication. *Shewanella putrefaciens* has been identified (Russell et al., 1996) as a common spoilage organism tied to poultry products similar to *Pseudomonas*. The odors that *Shewanella putrefaciens* produces has been described (Russell et al., 1996) as having a sulfur or "dishrag" odor notes. Dark, Firm and Dry (DFD) meat which has been shown (Doyle et al., 2009) to contain less glucose, tends to deteriorate at a rate that is more rapid due to bacteria like *Shewanella putrefaciens* resulting in less surface competition for microorganisms.

**Volatile organic compounds**

Most consumers are aware that off-odors are an indicator the meat product may have spoiled or, more specifically may be unwholesome for consumption. Volatile organic compounds (VOCs) are the culprits behind this off-odor (Pothakos et al., 2015) because they are the compounds created by microorganisms that consumers smell. This odor has a defect on sensory quality and will be rejected by consumers (Casaburi et al., 2015) which may be a dairy/cheesy smell or sulfur odor. The packaging and storage of meat will have an impact on the

growth of bacteria causing spoilage and leading to the consumer rejection of meat and food products. This is an important factor for storing meat products because the initial microbial (Casaburi et al., 2015) loading on the surface or within the meat product during manufacturing can lead to the bacteria producing a variety of VOCs. The types of VOCs that can be produced are alcohols, esters, aldehydes, ketones, and sulfur compounds. When a microorganism produces these off odors and flavonoids (in't Veld et al., 1996) they are referred to as a specific spoilage organism (SSO) within the meat and poultry industries.

## Strategies to Control Microbial Spoilage

### Packaging

Packaging of meat and poultry products can aid in protecting a product from contamination by creating a barrier to the environment. Two common methods of packaging in the poultry industry are bulk-packaged controlled vacuum packaging (CVP) and poly-vinyl chloride (PVC) film wrapped over an expanded polystyrene tray. Bulk-packaging of chicken products tend to be devoted to foodservice entities whereas, retail ready product tends to be linked to the PVC packaging applications. Bulk-packaging can maintain the weight of the packaged product by preventing (Seideman et al., 1983) shrink loss occurring through surface dehydration. Overwrap allows oxygen transmission across the physical barrier of the packaging material, which protects the packaged meat product from contamination, but allows for pathogens to grow and cause spoilage. In the poultry industry, overwrap allows oxygen in the package, which helps prevent the sulfur odor found in vacuum-sealed poultry.

When a piece of meat is vacuum sealed it can produce some odors (Seideman et al., 1983) such as cheesy and sour if the product is stored for extended periods of time. Sulfur odors

come from the anaerobic bacteria in the package (Casaburi et al., 2015) that are consuming amino acids that contain sulfur compounds (methionine and cysteine) during the storage period. Vacuum packaging is a method that removes the atmospheric gases from the pouch of meat and poultry products using a vacuum pump. Following atmospheric gas extraction, a top and bottom bar are heated to weld the packaging material closed. The pouch consists of a multilayer film that can contain two or more films. The films consist of resins, which are solid materials that are used to produce polymers. Packaging companies are capable of creating a variety of packages by combining different types of films. Normally the layers consist of ethyl vinyl acetate, polyvinylidene chloride and ethyl vinyl acetate (Zhou et al., 2010) and poultry that is packaged in an oxygen-impermeable package has been shown (Doyle et al., 2001) to be susceptible to spoilage from *Shewanella putrefaciens*, *B. thermosphacta,* and lactic acid bacteria. When the package is exposed to oxygen a green pigment called sulfmyoglobin is visible (Lambert et al., 1989) on the surface of the meat or poultry product.

Flat packaging is a typical method of packaging for the hotel, restaurant, institution (HRI) of the poultry industry, due to its quick and easy process of packaging and the likelihood of using a set amount within a hotel or restaurant kitchen. Flat packing is simply pushing air out of a package and then folding the package over to create a makeshift seal. Once it is packaged the product will move to a freezer and then shipped to the customer. Normally, this method is used for manufacturers that are sending marinated product to a customer from the hotel, restaurant or institution (HRI) segment of the industry, this form of packaged poultry product rarely makes it way to the retail consumer.

**Temperature**

A common method of food preservation is refrigeration, which keeps the meat cold and delays bacterial growth. Refrigeration (Barbut et al., 2015) can be broken into chilling (4 to 6ºC), freezing (-1 to -2ºC) and blast freezing (-190ºC) for meat and poultry products. It has been reported that (Zhou et al., 2010) chilling can affect the eating quality, shelf life and appearance of the meat. As the carcass chills the cold air will reduce carcass temperature and outer moisture of the carcass, which will help to prevent microbial growth due to the dry cold surface. Rapidly chilling meat and poultry pre-rigor can cause cold shortening (Zhou et al., 2010), resulting in tougher meat. Storing poultry products in temperatures ranging from 8 to 10ºC can be used to lengthen the shelf life of the products, but cold temperatures have been shown (Doyle et al., 2009) to aid in slowing bacterial growth.

Freezing meat products has been a practice since 1880 (Critchell et al., 1969; Arthur et al., 2006), when Australia shipped frozen beef to Britain. According to Barbut et al., (2015) freezing meat at temperatures below -12ºC can increase the shelf life of a product by inhibiting microbial growth. When meat is frozen the process can happen quickly or slowly. Fast freeze is the most efficient way to freeze meat for quality because it will create smaller ice crystals. If there are large ice crystals then they can damage cells, which will cause more drip when the product is thaws lowering the WHC (Zhou et al., 2010). Slow freeze creates large ice crystals, which can damage the microorganism's cell and hurt the growth potential of the bacteria (Doyle et al., 2009).

**Chemical**

Preservatives, such as organic acids, can inhibit bacterial growth of poultry products and when limiting bacterial growth sensory properties such as visual, odor and taste could be

improved. Organic acids decrease the muscle pH and a reduction in pH can create unfavorable conditions for microorganisms, which therefore prevents microorganism survival and growth. There is potential that the addition of an organic acid to a vacuum-sealed product could prevent the sulfur odor by eliminating the proliferation and survival of microorganisms causing this off odor as a byproduct of microorganism metabolism.

## Organic Acids Mechanisms

### Overview of Membrane Channels and Pumps

The cells of a microorganism innately desire to stay in equilibrium, and they do this through active transport and passive transport or facilitated diffusion (Doyle et al., 2001). Active transport requires pumps, such as the $Ca^{2+}$ pump, which hydrolyzes ATP to pump $Ca^{2+}$ into the sarcoplasmic reticulum (Alberts et al., 1989). Channel proteins located the lipid bilayer and carrier proteins allow ions to flow through the membrane in a downhill direction (passive transport) (Alberts et al., 1989). The sodium potassium ATPase is the pump that uses the hydrolysis of ATP to provide energy to pump sodium out and potassium into the cell (Berg et al., 2002). For every three $Na^{+}$ pumped out there are two $K^{+}$ pumped into the cell. This causes an unequal concentration of $K^{+}$ and there is a large negative charge inside the cell due to the fixed anions (Alberts et al., 1989). These fixed anions cannot cross the plasma membrane and therefore are confined to the inside of the cell (Lambert et al., 1998). The plasma membrane has a voltage gradient, which runs by keeping the inside more negative than the outside of the cell (Alberts et al., 1989). Knowing how the cell membrane works is important when looking at organic acids. Organic acids work on a cellular level to help inhibit bacteria.

**Organic acids**

Acetic and lactic acid are both weak-organic acids commonly used as food preservatives. Acetic acid is a monocarboxylic acid that has a strong smell and taste (Mani-Lopez et al., 2012), which limits its usage in meat products. Lactic acid is a monocarboxylic acid that is commonly produced (Axelsson, 2004) by bacteria through fermentation. Lactic acid can be directly applied to meat products and is a popular (Papadopoulos et al., 1991a, 1991b) preservative due to its capability to inhibit microbial growth, slow down lipid oxidation and prevent off-odor development. The two main mechanisms that organic acids use to inhibit pathogen growth is by the accumulation of the dissociated acid anion to toxic levels and by cytoplasmic acidification with subsequent uncoupling of energy production and regulation (Taylor et al., 2012). This does not kill the pathogens, but it does inhibit them to extend the shelf life of the meat product.

**Accumulation of Anions**

The normal pH for a meat product is 5.6 and bacteria are capable of maintaining a constant intracellular pH when the environment of the external pH is within 1 to 2 units (Carpenter et al., 2009) out of neutral muscle pH. Preservatives work more efficiently at an acidic pH value (Lambert et al., 1998) because the solution has a greater concentration of undissociated acids. Undissociated weak acids have the capability to diffuse through the pathogen's cell membrane (Stein, 1981) and they do this until equilibrium is reached. When the pH outside the microorganism's cell is lower than its internal pH, acid anions inside the cell will accumulate. The microorganism will accumulate potassium ions to generate turgor pressure which, will allow the cell to grow (Roe et al., 1998) but the increase in anion accumulation also increases the osmolarity of the cell, which could lead to a fatal turgor pressure (Carpenter et al.,

2009) causing the cell to rupture. Although osmolarity is increased, when the weak acid dissociates the dissociated anion is not permeable and cannot leave the cell (Lambert et al., 1998) The driving force for the accumulation of anions is the concentration of anions outside the cell and external pH (Carpenter et al., 2009) inhibiting growth.

Pathogens have an increased lag time of growth due to the presence of the organic weak-acid and ultimately causing an inhibition of (Lambert et al. 1998) microorganisms that are located on the surface of meat and poultry products. It has been reported that weak acids can impact internal postmortem meat pH and furthermore hinder the growth of microorganisms. Previous research by Lambert et al. (1998) reported that at lower muscle pH the enzyme $H^+$-ATPase was slow but as muscle pH (5.5) increased, the $H^+$-ATPase enzyme can be reacting at faster speeds. Research suggests (Lambert et al., 1998) that at a neutral pH the $H^+$-ATPase enzyme showed to have 70% of optimum activity. The addition of the weak acid will affect the pH causing the microorganisms to pump protons out of the cell to achieve a high enough pH to enter the growth phase (Lambert et al., 1998). The more acidic pH causes the cellular machinery responsible for taking in nutrients to slow down.

**Cytoplasmic Acidification**

The other mechanism that organics acids use to aid in preserving food products is cytoplasmic acidification. Cytoplasmic acidification (Carpenter et al., 2009) is when weak acids enter into the cytoplasm, where the acids can dissociate the liberating protons. Mani-Lopez et al., (2012) explains in a review that the cytoplasm pH declines, which will cause damage to enzymes, protein structures and DNA. The amount of acidification depends on the acid's pKa value, the pH gradient of the trans-membrane and the amount of the acid that is used (Ferguson

et al., 1995). Two other factors of cytoplasmic acidification are the potassium-proton and sodium-proton antiporters.

When the vesicles have a drop to a more acidic pH, (Booth, 1985) they can cause a negative impact on the antiporters. Acidic pH will cause the antiporters to be electroneutral while at an alkaline pH it will be electrogenic (Ramos et al., 1976; Raven et al., 1976) and so the antiporters exchange either potassium or sodium for protons to reach pH homeostasis. The $Na^+$ - $H^+$ exchange carrier is an antiport that removes excess $H^+$ ions to help prevent the acidification (Alberts et al., 1989). It is thought that in the absence of potassium or (Booth, 1985) when the concentration gradient is low that the sodium is exchanged for protons as an alternate route to achieve pH homeostasis.

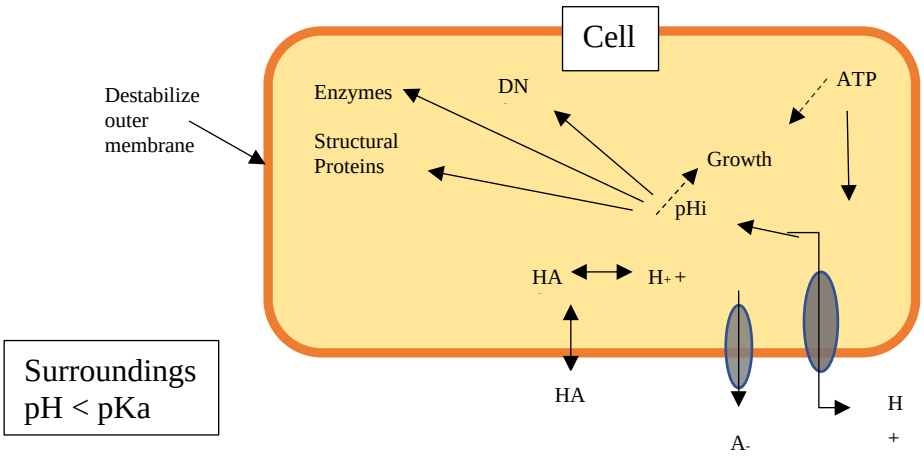



Figure 3. Action mechanism of organic acids on microbial cells. The undissociated form of organic acid (HA) is diffusing through the microbial membrane when the pH of the cellular cytoplasm is greater than that of the surrounding environment. In order to maintain the internal pH, active transport to efflux protons ($H^+$) is required. Also, acid pH in the internal cell, damages or modifies the functionality of enzymes, structural proteins, and DNA. *Few organic acids (malic and citric acids) have been shown to efficiently destabilize the outer membrane by chelation or intercalation.

(adapted from Mani-Lopez et al., 2012).

## Conclusion

The poultry industry produces a lot of product that is consumed within the United States and around the world. Since there is an increasing demand for more convenient consumer products, there has been a growth in further processing poultry facilities within the meat industry. The more a product is touched and processed, the greater likelihood that microbial loading can occur (Doyle et al., 2009) not only in the production facility but also within the manufactured product. It is important for manufacturing plants, restaurants and retail outlets to understand and identify the shelf life of their products or even better the initial microbial load. Testing of raw and finished poultry products for initial microbial load of a portioned product from start to finish can aid in developing prediction models for meat manufacturing companies. These models and timelines aid manufacturing companies in assisting their customers with a timeline of when the specific product will begin to exceed microbial spoilage thresholds and become unwholesome for consumers. Research has shown (Barbut et al., 2015) that a certain degree of microbial growth ($10^6$-$10^7$ CFU/g) creates signs of spoilage organism development. Although microorganism plating can give an indication of the microbial load, it is not the most accurate test. Consumers cannot see these microorganisms, but they tend to provide sensory factors that become noticeable by smelling and tasting of rancid flavonoids, which makes our senses a key aspect for detecting poultry spoilage. Again, the literature states that products are typically spoiled in the range of $10^6$-$10^8$ CFU/g but the reason they are actually considered spoiled is because at this point of microbial growth there is normally off odor and slime

production (Desai et al., 2014). Finally, by using microbial sampling methods, one could potentially find the shelf life of a product.

## Chapter II. Materials and Methods

### Chicken tenders

Fresh chicken tenders (48 h post-mortem) were hand trimmed, weighed and sorted. After portioning, 680.4 kg of tenders (226.8 kg/replication x 3 replications) were placed inside a plastic combo liner into 150 qt insulated coolers (Igloo, 105.75 cm x 47.48 cm x 51.44 cm, Katy, TX) with ice beneath a liner to keep the meat separated from the ice. After filling each cooler with chicken tenders, a ThermaData series II Temp Logger T2C (2 Ext. Removable Probes, American Fork, UT) temperature data logger was inserted into the chicken tenders in two different adjacent locations of the cooler to monitor product temperature during transportation from the processing facility. Chicken tenders were transported to the Lambert-Powell Meat Lab at Auburn University, and upon arrival were allocated randomly to 3 replications (226.8 kg per replication) and stored 48 h at 2 ºC in the absence of light. The test consisted of three replications and each of the three replications used 226.8 kg of hand trimmed chicken tenders.

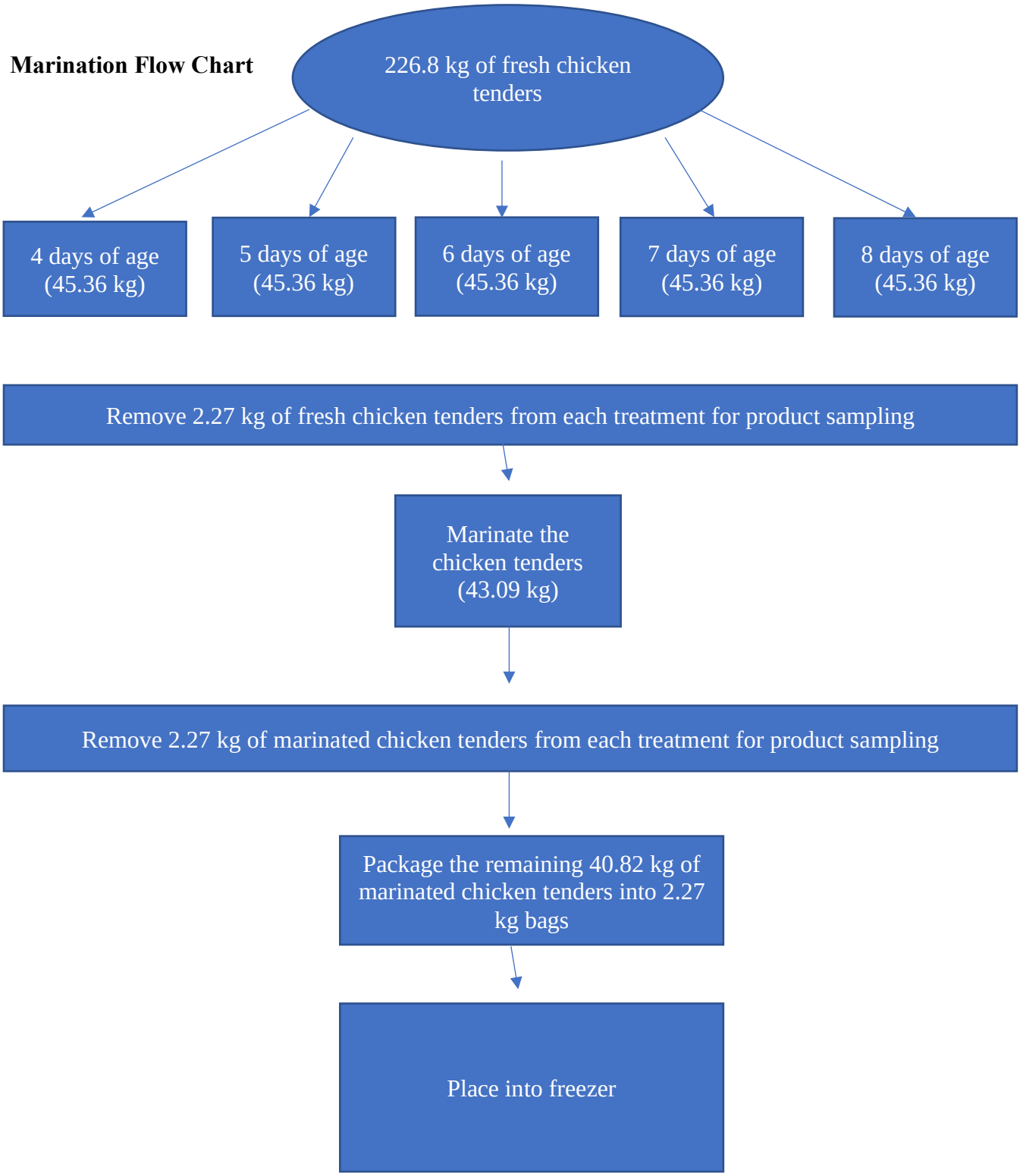
### Treatment Allocation

Chicken tenders (45.36 kg batch/treatment) from each replication (N = 3) were randomly allocated to one of five treatments (DA4, DA5, DA6, DA7, and DA8) based on storage time post-harvest. Batches (45.36 kg) of chicken tenders were placed into blue plastic bags (C and E Supply LLC, 13X20 + 1.5" LIP Blue Bag) (2.27kg/bag) and pressed flat by hand. Flat packing was achieved by pushing any remaining air out of the bag, folding the flap of the bag over and flipping the bag over on top of that flap with no actual seal. After flat packing, three bags from each treatment were randomly selected for microbial sampling of the marinated product prior to freezing.

**Marinating**

Before marinating 2.27kg of fresh chicken tenders were removed from the 45.36kg fresh chicken tenders for product sampling. At the time of processing, the remaining fresh chicken tenders (43.09 kg) per batch (N = 3) were allocated to vacuum tumbling marination. A proprietary marinade blend (1.64 kg) of ingredients which included salt, modified corn starch and monosodium glutamate was mixed with chilled water (5.79kg) for five minutes. After marinade mixing, chicken tenders and marinade solution were placed inside a vacuum tumbler (LT-30 Rotary Vacuum Tumbler 500 Pounds, Koch, Kansas City, Missouri) and atmosphere inside the tumbler was -25 Hg using a vacuum pump then subsequently tumbled at 4 rpm for 6 min.

**Marination Flow Chart**



**Product Sampling**

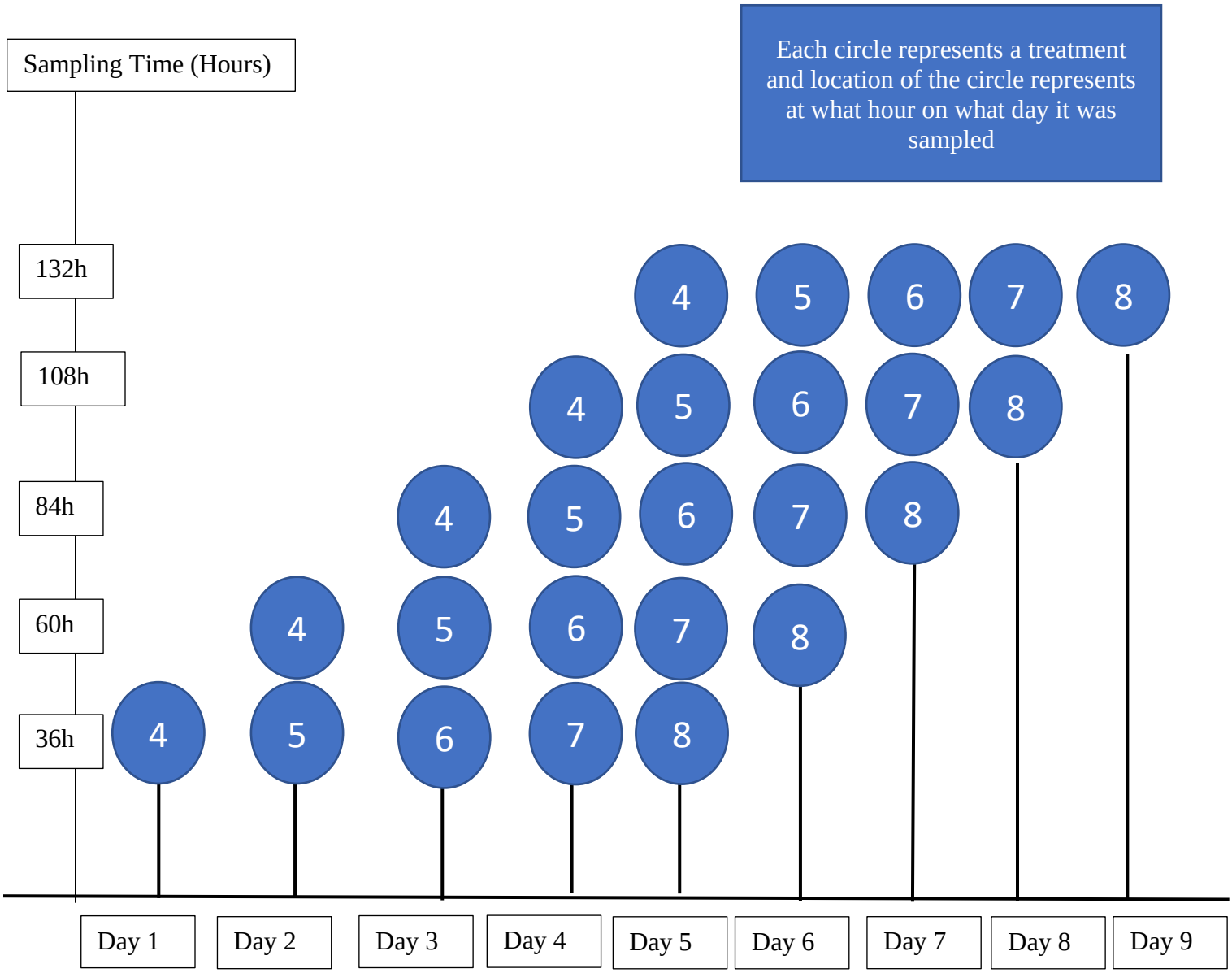
Fresh and marinated chicken tenders were sampled daily for microbial analysis. Every day of sampling, the temperature of the storage cooler was recorded. On the designated day of sampling, bags (n = 3) of bulk packaged (2.27 kg) fresh chicken tenders were identified for microbial sampling. From each bag, two tenders were aseptically removed and placed into a Whirl-pak bag (55 OZ. Filter Bag 7.5" X 12", Nasco, Fort Atkinson, WI) then transported to campus in a cold storage container. The remainder of the chicken tenders were marinated as described above. Following marination, chicken tenders were separated in to 2.27 kg allotments and placed inside blue plastic bags and flat packed. For sampling, two chicken tenders aseptically removed from the bag and placed into a Nasco whirl-pak bag for micro testing. From the remaining product in the bag. Illuminant D65 at 10° observance angle and a 3.5-cm aperture were utilized. The fifteen remaining, 2.27 kg bags of marinated chicken tenders were placed onto plastic trays and placed into the blast freezer (-25ºC) where they remained until all treatments were completed.

**Thaw**

The marinated chicken tenders remained frozen for 8 days and then were placed into a thaw in a walk-in cooler at that held at 4 ± 2°C for 36 h. Since each treatment was frozen on different days, they were also placed out to thaw on different days so that all samples were frozen for a total of 8 days (batch B and C). For ease of sampling, batch A was frozen for 20 days but followed the same thawing and sampling process. The thaw period was 36 hours in order to replicate the thawing specifications of the foodservice industry. The first sampling

period was 36 hours for color, micro, and pH. Following 36 hours, the sampling periods were every 24 hours over the next four days (60h, 84h, 108h and 132h).

**Thaw Flow Chart**



**Instrumental Color Analysis**

Fresh surface color was measured using the Commission International de'Eclairage (CIE) spectrum for lightness (L*), redness (a*) and yellowness (b*) values were evaluated using a HunterMiniscan XE Plus (MSXP-4500C; Hunter Laboratories, Reston, VA). Prior to each use, the colorimeter was first calibrated by using a black tile and white tile while enclosed in a polyethylene bag. Color measurements were measured in duplicate on the surface of the chicken tenders and then averaged.

**pH Analysis**

On each day of sampling, pH was measured using a probe style (H170 Hach pH meter, Hach, Loveland, Colorado) pH meter that was calibrated each day using a standard 4.0 and 7.0 buffer solution (Make Model Manufacturer) prior to collecting microbial and instrumental color samples. Chicken tenders (n = 2) were removed from their respective package and a stainless steel probe was inserted into the geometric center of each tender. Then the average was taken. Each treatment had three samples at every sampling period.

**Microbial Analysis**

Chicken tenders (n = 2) were aseptically removed from the 2.27 kg bag and placed into a Nasco Whirl-Pak filter bag for microbial analysis. The chicken tenders were rinsed with 50 ml of Phosphate Buffered Salt (PBS) and then massaged by hand for 1 min. After the rinsate, 1mL of solution from the Whirl-Pak filter bag was extracted with a filtered pipette tip and placed into a 10mL dilution tube containing 9 mL of PBS to create serial dilutions and mixed using an analog vortex mixer (VWR International, Radnor, PA). After serial dilution,100µL was extracted from

the dilution tubes or the whirl-pak bag and placed onto three different media types 1) aerobic plate (Difco$_{tm}$ Plate Count Agar) (incubated at 37ºC for one day), 2) psychotrophic plate (Difco$_{tm}$ Plate Count Agar)(incubated at 8ºC for 7 days) and 3)de Man Rogosa and Sharpe agar (MRS) (Difco$_{tm}$ Lactocacilli MRS Agar) (incubated in anaerobic chamber at 37ºC for two days). For MRS agar plates, the anaerobic chambers (MGC AnaeroPack® System, Rectangular Jar 7.0 L; Mitsubishi Gas Chemical Co., Inc., Chiyoda, Tokyo, Japan) contained two oxygen scavenger packs (GasPak™ EZ; Becton, Dickinson and Company, Sparks, MD, USA). For each plate type there were 3 dilutions plated per sample. For every sample collected each had a total of nine plates (3 aerobic, 3 psychotrophic and 3 MRS). After the incubation period plates colonies on each plate were counted on a Reichert Quebec Darkfield Colony Counter (Depew, New York) and recorded. The best plate was taken from each sample and then converted to CFU per mL of rinsate.

**Statistical Analysis**

There was a total of three batches with each sample tested in triplicate. Each sample had a total of nine plates per plate type (three types of plates) resulting in twenty-seven plates total. Microbial data was converted to $\log_{10}$ CFU/mL rinsate before statistical analysis was conducted. Using a repeated measures design, each media type, pH, and instrumental color were dependent variables and day of age and thaw period were fixed effects. The data were analyzed using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina) and LSMEANS separated using the Tukey-Kramer adjustment ($\alpha = 0.05$).

# Chapter III Results

## Initial Bacterial Levels

No treatment reached 6 log in this experiment, but a few began to approach the threshold. The earlier treatments Days of age (DA) 4 through 6 aerobic and Lactic Acid Bacteria (LAB) counts (fresh) were less ($P < 0.05$) than DA7 and DA8 (Table 1 and 2). DA8 had the greatest pyschotrophic count at the fresh sampling period and was greater ($P < 0.05$) than the other treatments. The greatest increase in the initial microbial load ($P < 0.05$) for MRS and aerobic bacteria occured at DA7, while psychotrophic bacteria have the greatest increase at DA8.

## Marination Results

The marination process consisted of the addition of a marinade (containing a proprietary blend of salt, modified corn starch, and monosodium glutamate), and tumbling in a vacuum for six minutes, which could inhibit the growth of some bacteria (Galarz et al., 2016; Jahani et al., 2018). The number of aerobic bacteria present in samples, declined for all treatments post marination. This was also observed in the pyschotrophic bacteria except for treatment DA4, which had a slight increase in psychotrophic bacteria (Table 3). Post marination (0 hours), the microbial load of psychotrophic and aerobic bacteria in DA4, DA5 and DA6 were less ($P < 0.05$) than DA7 and DA8 (Table 3).

## Thaw Test Results

The fast food restaurant chain that this particular experiment was working with, has specifications that allow the chicken to thaw for a maximum of 36h to batter, bread, cook and sell the chicken tenders. Allowing the chicken tenders to thaw gives an opportunity for psychotrophic bacteria to grow with little competition due to the temperature being low (Barbut

et al., 2015). The psychotrophic bacteria in each treatment began above log 3 CFU/mL but DA4 was less ($P < 0.05$) than the other treatments. Throughout the sampling periods the psychotrophic growth steadily increased for each treatment. At 60h Treatments DA4 and DA5 have a larger count ($P < 0.05$) of psychotrophic bacteria with the largest increase at 108h (approaching 5 log CFU/mL). Figures 4, 5 and 6 show the trends of the bacterial growth.

# Chapter IV Discussion

## Discussion

There are multiple steps that a portioning facility can take in order to prevent microbial growth. During the manufacturing of fresh chicken parts and pieces it is important to extend the lag phase of the bacterial growth curve because the microorganisms are not growing exponentially. Most commercial processing facilities currently use an organic acid throughout many phases of poultry processing from deboning and trimming to further processing and marinating the product. Organic acids are a common practice for food safety and could possibly contribute to the initial microbial loads witnessed during the current study (Barbut et al., 2015; Desai et al., 2014). The treatments stored for a longer period of time, DA7 and DA8, resulted in greater Lactic Acid Bacteria (LAB) (Table 2). These types of bacteria favor lower to no oxygen conditions and these two treatments were at the bottom of the combo, which is where the least expected amount of oxygen would be. The chicken tenders also went through a peracetic acid rinse which lowers the pH of the chicken tenders and (Pothakos et al., 2015) could be a more favorable environment for LAB.

Additionally, aerobic and psychotrophic (except for DA4) microbial loads for the chicken tenders declined following marination with a water, salt, modified food starch and monosodium glutamate. It is plausible that the addition of the marinade ingredients resulted in lowering the surface pH of the chicken tenders causing microorganism growth to be limited. Galarz et al., (2016) compared raw chicken breasts' microbial loads to salted chicken breasts' microbial loads and found that the salted chicken breasts had lower amounts of bacteria. Also, the addition of the wet marinade could have had a diluting effect on the microbial load. Immediately after marintating, the chicken tenders were packaged and placed into a blast freezer (-24ºC). In a

review by Dave et al., (2011), freezing can prevent 60% of the bacterium from growing. The extremely cold temperature can cause injury to the bacterial cells which would require favorable storage conditions (temperature) for bacteria repair (Dave et al., 2011).

Psychotrophs had the greatest microbial level throughout this study because of the colder storage temperatures maintained during the test. It appears that as the chicken tender product thawed, the psychotrophs were first to reach a favorable temperature for growth and repair. The psychotrophic bacteria grow faster in colder temperatures (0 to 20ºC), while mesophilic bacteria grow faster in warmer temperatures (20 to 45ºC). A shelf life experiment conducted by Galarz et. al, (2016), compared how different temperatures (2ºC, to 20ºC) affected the growth of psycotrophic and mesophilic aerobic bacteria on portions of chicken breast. The psychotrophic microbial load was greater than the aerobic bacetria at the initial and last sampling periods (Galarz et al., 2016), proving that psychotrophs will maintain greater counts at colder temperatures.

In the literature, Barbut et al., (2015), describes that at low refrigeration temperatures, psychotrophic bacteria will dominate the competition and that mesophiles may survive the cold conditions but not grow. Galarz et al., (2016) and Pothakos et al., (2015) explain that the greatest influencer of microorganism growth is microbial competition. Typically, bacteria will use glucose as the first nutrient to metabolize and reproduce. *Pseudomonas spp.* is a very common specific spoilage organism that is an aerobic psychotroph. The bacterium is a common culprit in the poultry spoilage realm (Stellato et al., 2017) and the environment of this study favors bacteria like *Pseudomonads* and other aerobic pyschotrophs. The rancid odors and slime production are signs of spoilage that attributed to bacteria like *Psuedomonads* (Blackburn et al., 2006) and can often be detected through sensory analytical methods. According to Doyle et al., (2009) lactic

acid bacteria do not create as strong off odors as the psychotrophic bacteria, which shows that sensory qualities are an important aspect of spoilage.

In conclusion, none of the bacterial counts reached 6 log CFU/mL, but when strong organoleptic changes occur via spoilage organisms a consumer will not consume the product. Consumers mentally process their own measurement of sensory aspects (Doyle et al., 2009) and decide whether the food is fit for consumption. These products that are not fit for consumption are not eaten and go to waste, which leads to profit loss. Chicken tenders DA7 and DA8 tended to have an easily detectable off odor, but this qualitative measurement was not conducted during the current study. The results of this current study suggest that chicken tenders manufactured using these same techniques (Fresh, Marinated, Frozen, Thawed) are capable of achieving an eight day shelf life before reaching the microbial spoilage threshold (6 log) widely considered within the poultry industry. With an increase in spoilage organisms, it is plausible that odor production will occur. Furthermore, thawing of frozen chicken tenders suggests that the age post harvest will not impact the duration of storage that thawed chicken tenders could be used in a foodservice setting. Chicken tenders thawed after frozen storage did not reach a 6 log spoilage threshold and could be considered for use in a restaurant setting. It does appear that treatments DA4, and DA5 begin to grow more rapidly at 108h for psychotrophic bacteria and 60h for aerobic bacteria. Desai et al., (2014) explains in their study that the chicken breast they sampled was approaching spoilage when the microbial load was 5.2 log CFU/g and that a couple of days later the product was past the threshold.

The facilities current operation appears to minimize spoilage bacteria due to processing techniques and which could lead to extensions of their current shelf life for their chicken tender products. Consumer satisfaction is the most important priority for food service (Khan et al., 2013)

because through satisfaction, consumer loyalty can develop. Although, a reputation can be ruined by one bad experience causing potential loss in profit for the restaurant. Sensory qualities such as off odor and slime can hurt the consumer's perception of the meat product (Font-i-Furmos et al., 2014) leading to a consumer deciding that the meat is unacceptable. Finally, a better understanding of a meat product's shelf life can lead to better experiences for consumers by preventing unwanted odors.

**Future Research**

Additional research regarding foodservice chicken tenders could focus on the duration of storage and sampling periods. It would be interesting to evaluate time from bird harvest as well as extending the post thaw period. Through extending the days from harvest from that was capped at 8, the inclusion of an additional 3 days could provide worthy answers to foodservice chicken tender shelf life. These potential research questions could provide a better understanding of microorganism growth that occurs in fresh chicken intended for foodservice entities. Moreover, it would be beneficial to examine fresh versus marinated chicken in an effort to identify the influence of further processing on fresh chicken tender shelf life in a foodservice simulation of fresh, thaw, fresh.

Literature Cited

Ahn, D.U., Wolfe, F. H., and Sim, J.S., and Kim, D. H. "Packaging Cooked Turkey Meat Patties While Hot Reduced Lipid Oxidation." *Journal of Food Science* 57 (1992):1075-1077, 1115.

Alberts, Bruce, et al. *Molecular Biology of the Cell.* Second ed., Garland Science, 1989.

Allen, C. D., S. M. Russell, and D.L. Fletcher. "The relationship of broiler breast meat color and pH to shelf-life and odor development." *Poultry Science* 76, no. 7(1997): 1042-1046.

Arthur, Ian. "Shipboard refrigeration and the beginnings of the frozen meat trade." *Journal of the Royal Australian Historical Society* 92, no. 1 (2006): 63.

Axelsson, Lars. "Lactic acid bacteria: classification and physiology." *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER*- 139 (2004): 1-66.

Ayres, J. C. "The relationship of organisms of the genus Pseudomonas to the spoilage of meat, poultry and eggs." *Journal of Applied Bacteriology* 23, no. 3 (1960): 471-486.

Bagge, Dorthe, et al. "Shewanella putrefaciens adhesion and biofilm formation on food processing surfaces." *Appl. Environ. Microbiolgy.* 67, no. 5 (2001): 2319-2325.

Barbut, Shai. "*The Science of Poultry and Meat Processing"*. 2015.

Barbut, S., L. Zhang, and M. Marcone. "Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets." *Poultry science* 84, no. 5 (2005): 797-802.

Barbut, S. "Pale, soft, and exudative poultry meat- Reviewing ways to manage at the processing plant." *Poultry science* 88, no.7 (2009): 1506-1512.

Barnes, Ella M., and Wendy Melton. "Extracellular enzymic activity of poultry spoilage bacteria." *Journal of Applied Bacteriology* 34, no.3 (1971): 599-609.

Barnes, Ella M., and C. S. Impey. "Psychrophilic spoilage bacteria of poultry." *Journal of Applied Bacteriology* 31, no. 1 (1968): 97-107.

Berg, Jeremey M, et al. "A Family of Membrane Proteins Uses ATP Hydrolysis to Pump ions Across Membranes." *Current Neurology and Neuroscience Reports.,* U.S. National Library of Medicine, 1 Jan. 1970, www.ncbi.nlm.nih.gov/gov/books/NBK22464/.

Blackburn, Clive de W. "Food spoilage microorganisms, edited by Clive de W. Blackburn." *Woodhead Publishing in Food Science, Technology and Nutrition.* (2006).

Blaszyk, Maria, and Richard A. Holley. "Interaction of monolaurin, eugenol and sodium citrate

on growth of common meat spoilage and pathogenic organisms." *International journal of food microbiology* 39, no. 3 (1998): 175-183.

Booth, Ian R. "Regulation of cytoplasmic pH in bacteria." *Microbiological reviews* 49, no. 4 (1985):359.

Brul, S, and P Coote. “Preservative Agents in Foods Mode of Action and Microbial Resistance Mechanisms.” *International Journal of Food Microbiology,* 50, no. 1-2 (1999): 1-17.

Buzby, Jean C., Hodan Farah Wells, and Jaspreet Aulakh. *Food loss—questions about the\ amount and causes still remain*. No. 1490-2016-128501. 2014.

Carpenter, C. E., and Jeffery R. Broadbent. "External concentration of organic acid anions and pH: key independent variables for studying how organic acids inhibit growth of bacteria in mildly acidic foods." *Journal of food science* 74, no. 1 (2009): R12-R15.

Casaburi, Annalisa, et al. "Bacterial populations and the volatilome associated to meat spoilage." *Food microbiology* 45 (2015): 83-102.

Chen, T.C., & Waimaleongora-EK, C. (1981). “Effect of pH on TBA Values of Ground Raw Poultry Meat.” *Journal of Food Science,* 46 (1981): 1946-1947.

Critchell, J. T., & Raymond, J. A history of the frozen meat trade. London: Dawsons of Pall Mall. 1969

Dashdorj, Dashmaa, et al. “Dry aging of beef; Review.” *Journal of animal science and technology* 58, no.1 (2016):20.

Dave, D., and Abdel E. Ghaly. "Meat spoilage mechanisms and preservation techniques: a critical review." *American Journal of Agricultural and Biological Sciences* 6, no.4 (2011): 486-510.

Davis, Gregg S., et al. “Antibiotic-resistant Escherichia coli from retail poultry meat with different antibiotic use claims.” *BMC* microbiology 18, no. 1 (2018): 174.

Decker, E. A., & Mei, L.Y. “Antioxidant Mechanisms and Applications in Muscle Foods.” *Reciprocall Meat Conference Proceeding,* 49 (1996): 64-72.

del Río, Elena, et al. "Effect of poultry decontaminants concentration on growth kinetics for pathogenic and spoilage bacteria." *Food microbiology* 25, no.7 (2008): 888-894.

Desai, Monil A., et al. "Utilization of buffered vinegar to increase the shelf life of chicken retail cuts packaged in carbon dioxide." *Poultry science* 93, no.7 (2014): 1850-1854.

Dominguez, Silvia A., and Donald W. Schaffner. “Development and validation of a mathematical model to describe the growth of Pseudomonas spp. in raw poultry stored

under aerobic conditions." *International journal of food microbiology* 120, no.3 (2007): 287-295.

Douglas, Roseileen M., et al. "The distribution of homologues of the Escherichia coli KefC K+-efflux system in other bacterial species." *Microbiology* 137, no.8 (1991): 1999-2005.

Doulgeraki, Agapi I., et al. "Spoilage microbiota associated to the storage of raw meat in different conditions." *International journal of food microbiology* 157, no.2 (2012): 130-141.

Doyle, Michael P. *Compendium of the microbiological spoilage of foods and beverages.* Springer Science & Business Media, 2009.

Doyle, Michael P., et al. *Food Microbiology Fundamentals and Frontiers.* 2nd edition., ASM Press, 2001.

Eklund, T. "The Effect of Sorbic Acid and Eters of *Para*-hydroxybenzoic acid on the Proton Motive Force in *Eschericia coli* Membrane Veslicles." *Journal of General Microbiology.* 131 (1985):73-76.

Ferguson, Gail P., Debra McLaggan, and Ian R. Booth. "Potassium channel activation by glutathione-S-conjugates in Escherichia coli: protection against methylglyoxal is mediated by cytoplasmic acidification." *Molecular microbiology* 17, no.6 (1995): 1025-1033.

Font-i-Furnols, Maria, and Luis Guerrero. "Consumer preference, behavior and perception about meat and meat products: An overview." *Meat science* 98, no. 3 (2014): 361-371.

Foster, J.W., and Hall, H.K. "Inducible pH Homeostasis and the Acid Tolerance Response of Salmonella Typhimurium." *J Bacteril* 173 (1991): 5129-5135.

Francis, A. J., Dodge, C.J., & Gillow, J. B. "Biodegradation of Metal Citrate Complexes and Implications for Toxic-Metal Mobility." *Nature,* 356 (1992):140-142.

"FSIS." *Poultry Products Inspection Acts,* www.fsis.usda.gov/ppia. Jan. 21, 2016

Galarz, Liane Aldrighi, Gustavo Graciano Fonseca, and Carlos Prentice. "Predicting bacterial growth in raw, salted, and cooked chicken breast fillets during storage." *Food Science and Technology International* 22, no.6 (2016): 461-474.

Gale, E.F., and Epps, H.M.R "The Effect of the pH of the Medium During Growth on the Enzymic Activities of Bacteria (E. coli and M. lysodiekticus) and the Biological Significance of the Changes Produced." *Biochemistry* 36 (1942): 600-619.

Gennari, Mario, et al. "Isolation and characterization by conventional methods and genetic

transformation of Psychrobacter and Acinetobacter from fresh and spoiled meat, milk and cheese." *International journal of food microbiology* 15, no.1-2 (1992): 61-75.

Gram, Lone, and Jette Melchiorsen. "Interaction between fish spoilage bacteria Pseudomonas sp. and Shewanella putrefaciens in fish extracts and on fish tissue." *Journal of applied bacteriology* 80, no.6 (1996): 589-595.

Gratta, F., et al. "Effect of breast myopathies on quality and microbial shelf life of broiler meat." *Poultry science* 98, no.6 (2019): 2641-2651.

Helander, Ilkka M., et al. "Characterization of the action of selected essential oil components on Gram-negative bacteria." *Journal of agricultural and food chemistry* 46, no.9 (1998): 3590-3595.

Hirasa, K. "Antimicrobial and antioxidant properties of spices." *Spice science and technology* (1998).

Hopper, D.J., and Cooper, R.A. “The Regulation of *Escherichia coli* Methylglyoxal Synthase.” *FEBS Letts* 13 (1971):213-216.

Hopper, D.J., and Cooper, R.A. “The Purification and Properties of *Escherichia coli* Methylglyoxal Synthase.” *Journal of Biochemistry* 218 (1972): 321-329.

in't Veld, Jos HJ Huis. "Microbial and biochemical spoilage of foods: an overview." *International journal of food microbiology* 33, no.1 (1996): 1-18.

Jahani, R., Z. Hamidi, and H. Ahmadi. "PSV-19 Effect of Pressure and Rotation Speed of Tumbling on Shelf-Life of Chicken Breast in Vacuum Tumbling." *Journal of Animal Science* 96, no.3 (2018): 203.

Kanner, J. "Oxidative processes in meat and meat products: quality implications." *Meat science* 36, no. 1-2 (1994): 169-189.

Khan, Shahzad, Syed Majid Hussain, and Fahad Yaqoob. "Determinants of customer satisfaction in fast food industry a study of fast food restaurants Peshawar Pakistan." *Studia commercialia Bratislavensia* 6, no. 21 (2013): 56-65.

Kilcher, Samuel, Martin J. Loessner, and Jochen Klumpp. "Brochothrix thermosphacta bacteriophages feature heterogeneous and highly mosaic genomes and utilize unique prophage insertion sites." *Journal of bacteriology* 192, no.20 (2010): 5441-5453.

Kockzon, P. “Growth Inhibition Mode of Action of Selected Benzoic Acid Derivatives Against the Yeast Pichia Anomala.” *Journal of Food Protection,* 72 (2009):791-800.

Krebs, H.A., Wiggins, D., Sole, S., Bedoya, F., "Studies on the Mechanism of the Antifungal Action of Benzoate." *Journal of Biochemistry.* 214 (1983): 657-663.

Kroll, R.G., and I.R. Booth. "The Role of Potassium Transport in the Generation of a pH Gradient in *Escherichia coli*." *Journal of Biochemistry* 198 (1981):691-698.

Lambert, Anne D., James P. Smith, and Karen L. Dodds. "Shelf life extension and microbiological safety of fresh meat – a review." *Food Microbiology* 8, no.4 (1991): 267-297.

Lambert, R. J., and M. Stratford. "Weak-acid preservatives: modelling microbial inhibition and response." *Journal of applied microbiology* 86, no.1 (1999): 157-164.

Ladikos, D. and Lougovois, V. "Lipid Oxidation in Muscle Foods: A Review." *Food Chem.* 35 (1990):295-314.

Mani-López, E., H. S. García, and A. López-Malo. "Organic acids as antimicrobials to control Salmonella in meat and poultry products." *Food Research International* 45, no.2 (2012): 713-721.

McDonald, Karl, and Da-Wen Sun. 'Predictive food microbiology for the meat industry: a review." *International journal of food microbiology* 52, no.1-2 (1999): 1-27.

Meury, J., Lebail, S., and Kepes, A. "Opening of Potassium Channels in *Escherichia coli* Membranes by Thiol Reagents and Recovery of Potassium Tightenss." *Eur. J. Biochem.* 113 (1980): 33-38.

Moser, Duane P., and Kenneth H. Nealson. "growth of the facultative anaerobe Shewanella putrefaciens by elemental sulfur reduction." *Appl. Environ. Microbiol.* 62, no.6 (1996): 2100-2105.

Nakamura, Tatsunosuke, Hajime Tokuda, and Tsutomu Unemoto. "K+/H+ antiporter functions as a regulator of cytoplasmic pH in a marine bacterium, Vibrio alginolyticus." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 776, no.2 (1984): 330-336.

Nam, Ki-Chang. "Mechanisms of color change and the prevention of off-color and off-flavor in irradiated meat." (2002).

Newton, K. G., and C. O. Gill. "The development of the anaerobic spoilage flora of meat stored at chill temperatures." *Journal of Applied Bacteriology* 44, no.1 (1978): 91-95.

Nychas, George-John E., et al. "Meat spoilage during distribution." *Meat science* 78.1-2 (2008): 77-89.

Ogden, S.K., Gurrero, I., Taylor, A.J., Buendia, H.E., & Gallardo, F. “Changes in Odour, Color and Texture During the Storage of Acid Preserved Meat.” *Lebensmittel-Wissenschaft und-Technologie,* 28 (1995): 521-527.

Papadopoulos, L.S., Miller, R.K. Acuff, G.R., Lucia, L.M. Vanderzant, C., & Cross, H.R. “Consumer and Trained Sensory Comparisons of Cooked Beef Top Rounds Treated with Sodium Lactate.” *Journal of Food Science,* 56 (1991a): 1141-1146.

Papadopoulos, L.S., Miller, R.K. Acuff, G.R., Lucia, L.M. Vanderzant, C., & Cross, H.R. “Effect of Sodium Lactate on Microbial and Chemical Composition of Cooked Beef During Storage.” *Journal of Food Science,* 56 (1991b): 341-347.

Park, Yong-Keun, et al. "Internal pH crisis, lysine decarboxylase and the acid tolerance response of Salmonella typhimurium." *Molecular microbiology* 20.3 (1996): 605-611.

Parlapani, Foteini F., et al. "Microbiological spoilage and investigation of volatile profile during storage of sea bream fillets under various conditions." *International journal of food microbiology* 189 (2014): 153-163.

Parlapani, Foteini F., et al. "Microbiological spoilage and volatiles production of gutted European sea bass stored under air and commercial modified atmosphere package at 2 C." *Food microbiology* 50 (2015): 44-53.

Parlapani, Foteini F., et al. "The dynamics of Pseudomonas and volatilome during the spoilage of gutted sea bream stored at 2 C." *Food Control* 55 (2015): 257-265.

Pothakos, Vasileios, et al. "Lactic acid bacteria and their controversial role in fresh meat spoilage." *Meat science* 109 (2015): 66-74.

Ramos, S., And H.R. Kaback. “The Electrochemical Proton Gradient in *Escherichia coli* Membrane Vesicles.” *Biochemistry.* 16 (1976):848-853.

Raven, J.A., and F.A. Smith.“The Evolution of Chemiosmotic Energy Coupling.” *Journal of Theor. Of Biol.* 57 (1976):301-312.

Richard, Hope, and John W. Foster. "Sodium regulates Escherichia coli acid resistance, and influences GadX-and GadW-dependent activation of gadE." *Microbiology* 153, no.9 (2007): 3154-3161.

Roe, Andrew J., Debra McLaggan, Ian Davidson, Conor O’Byrne, and Ian R. Booth. "Perturbation of anion balance during inhibition of growth of Escherichia coli by weak acids." *Journal of bacteriology* 180, no. 4 (1998): 767-772.

Russell, S. M., D. L. Fletcher, and N. A. Cox. "Spoilage bacteria of fresh broiler chicken carcasses." *Poultry Science* 74, no.12 (1996): 2041-2047.

Salmond, C.V., Kroll, R.G., and Booth, I.R. “The Effect of Food Preservatives on pH Homeostasis.” *Journal of General Microbiology* 130, no.11 (1984): 2845-2850.

Seideman, S. C., and P. R. Durland. "Vacuum packaging of fresh beef: A review." *Journal of Food Quality* 6, no.1 (1983): 29-47.

Senter, Samuel D., Judy W. Arnold, and Victor Chew. "APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13 C and under simulated temperature abuse conditions." *Journal of the Science of Food and Agriculture* 80, no.10 (2000): 1559-1564.

Ke, Shuming, et al. "Impact of citric acid on the tenderness, microstructure and oxidative stability of beef muscle." *Meat science* 82, no.1 (2009): 113-118.

Smolinska, T., and M. Korzeniowska. “Evaluation of the PSE and DFD abnormalities occurrence in the chicken meat.” *Proc. XVII EUR. Symp. Poult. Meat, The Netherlands. World’s Poult. Sc. Assoc., Doorweth, the Netherlands.* 2005.

Stein, W. D. "Permeability for lipophilic molecules." *New Comprehensive Biochemistry*. Vol. 2. Elsevier, 1981. 1-28.

Stellato, Giuseppina, et al. "A few Pseudomonas oligotypes dominate in the meat and dairy processing environment." *Frontiers in microbiology* 8 (2017): 264.

Taylor, T. Matthew, et al. "13 Alternatives to Traditional Antimicrobials for Organically Processed Meat and Poultry." *Organic meat production and processing* 53 (2012).

Thakur, B.R. And Singh, R.K. 1994. “Food Irradiation: Chemistry and Applications.” *Food Rev. Int.* 10:437-473.

Tichivangana, J.Z., and P. A. Morrissey. “The influence of pH on lipid oxidation in cooked meats from several species.” *Irish Journal of Food Science and Technology* (1985): 99-106

Vinella, D. A. N. I. E. L., et al. "Penicillin-binding protein 2 inactivation in Escherichia coli results in cell division inhibition, which is relieved by FtsZ overexpression." *Journal of bacteriology* 175, no.20 (1993): 6704-6710.

Woelfel, R. L., et al. “The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant.” *Poultry science,* 81.4 (2002): 579-584.

Zhou, G. H., X. L. Xu, and Yuan Liu. "Preservation technologies for fresh meat–A review." *Meat science* 86, no.1 (2010): 119-128.

“Sustainable Management of Food Basics.” *EPA*, Environmental Protection Agency, 13 Nov. 2019, www.epa.gov/sustainable-management-food/sustainable-management-food-basics.

"USDA/NASS Poultry Production and Value 2018 Summary", *NASS*, National Agricultural Statistics Service. May 2019, www.nass.usda.gov/Publications/Todays_Reports/reports/plva0519.pdf.

## Tables and Figures

| Table 1. Interactive effects (Day x Time) on Aerobic plate count (APC)[1] of chicken tender during simulated foodservice shelf life | | | | | | | |
|---|---|---|---|---|---|---|---|
| Age of Chicken[3] | Thaw Time[2] (h) | | | | | | |
| | Fresh | 0 h | 36 h | 60 h | 84 h | 108 h | 132 h |
| 4 Days | 1.42$^{klm}$ | 0.662$^{n}$ | 0.727 $_{mn}$ | 1.62 $_{ijkl}$ | 2.9 $_{cd}$ | 2.72 $_{cdef}$ | 2.96 $_{bc}$ |
| 5 Days | 1.63 $_{ijkl}$ | 1.47 $_{jkl}$ | 0.971 $_{lmn}$ | 2.62 $_{cdefg}$ | 2.28$_{cdefghi}$ | 2.38 $_{cdefg}$ | 1.95 $_{ghijk}$ |
| 6 Days | 1.65 $_{hijkl}$ | 1.19 $_{lmn}$ | 2.54 $_{cdefg}$ | 2.34 $_{cdefgh}$ | 2.22 $_{defghi}$ | 1.51 $_{jkl}$ | 2.25 $_{defghi}$ |
| 7 Days | 3.81 $_{a}$ | 3.62 $_{ab}$ | 2.04 $_{fghijk}$ | 2.95 $_{bc}$ | 1.52 jkl | 2.03 $_{ghijk}$ | 2.46 $_{cdefg}$ |
| 8 Days | 2.82 $_{cde}$ | 2.58 $_{cdefg}$ | 2.14 $_{efghij}$ | 2.06 $_{fghijk}$ | 2.08 $_{fghijk}$ | 1.99 $_{ghijk}$ | 2.08 $_{fghijk}$ |

[1]Colony forming units (CFU)/g of sampled chicken tenders.
[2]Sampling period following fresh and frozen storage of packaged chicken tenders.
[3] Storage period of chicken elapsed following harvest.
[a,b,c,d,e,f,g,h,i,j,k,l,m,n]Means lacking common superscripts differ (P < 0.05).

| Table 2. Interactive effects (Day x Time) on Lactic Acid Bacteria Plate Count (APC)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|
| Age of Chicken[3] | Thaw Time[2] (hours) | | | | | | |
| | Fresh | 0 h | 36 h | 60 h | 84 h | 108 h | 132 h |
| 4 Days | 0.909 | 0.34 | 1.14$_{n}$ | 1.84$_{ijklmn}$ | 3.02$_{cdefg}$ | 2.69$_{efgh}$ | 3.82$_{abc}$ |
| 5 Days | 0.997 | 1.06$_{n}$ | 1.97$_{hijklm}$ | 1.72$_{jklmn}$ | 1.06$_{n}$ | 3.77$_{abcd}$ | 1.31$_{mn}$ |
| 6 Days | 0.253 | 0.496 | 1.35$_{mn}$ | 1.62$_{lmn}$ | 2.31$_{ghijkl}$ | 2.77$_{efgh}$ | 1.39$_{mn}$ |
| 7 Days | 3.19$_{bcdef}$ | 2.52$_{fghij}$ | 1.65$_{klmn}$ | 2.45$_{fghijk}$ | 3.49$_{bcde}$ | 2.38$_{ghijkl}$ | 4.39$_{a}$ |
| 8 Days | 3.05$_{cdefg}$ | 2.84$_{efg}$ | 3.01$_{cdefg}$ | 2.99$_{defg}$ | 1.25$_{mn}$ | 3.99$_{ab}$ | 2.61$_{fghi}$ |

[1]Colony forming units (CFU)/g of sampled chicken tenders.
[2]Sampling period following fresh and frozen storage of packaged chicken tenders.
[3] Storage period of chicken elapsed following harvest.
[a,b,c,d,e,f,g,h,i,j,k,l,m,n]Means lacking common superscripts differ (P < 0.05).

| Table 3. Interactive effects (Day x Time) on Psychotrophic Plate Count (PPC)[1] of chicken tender during simulated foodservice shelf life | | | | | | | |
|---|---|---|---|---|---|---|---|
| Age of Chicken[3] | Thaw Time[2] (hours) | | | | | | |
| | Fresh | 0 h | 36 h | 60 h | 84 h | 108 h | 132 h |
| 4 Days | 3.07 | 3.16 | 3.16 | $3.69_{hijk}$ | $4.06_{def}$ | $4.95_{b}$ | $5.26_{a}$ |
| 5 Days | $3.39_{lmn}$ | 3.15 | $3.24_{n}$ | $3.62_{ijkl}$ | $4.2_{cd}$ | $4.88_{b}$ | $5.07_{ab}$ |
| 6 Days | $3.21_{n}$ | 3.09 | $3.27_{mn}$ | $3.4_{klmn}$ | $3.8_{efghi}$ | $3.96_{defgh}$ | $4.4_{c}$ |
| 7 Days | $3.63_{ijkl}$ | $3.53_{ijklm}$ | $3.39_{lmn}$ | $3.69_{hijk}$ | $3.71_{ghij}$ | $4.19_{cd}$ | $4.08_{de}$ |
| 8 Days | $4.22_{cd}$ | $3.78_{fghi}$ | $3.47_{jklmn}$ | $3.65_{ijkl}$ | $3.64_{ijkl}$ | $4_{defg}$ | $4.1_{cd}$ |

[1]Colony forming units (CFU)/g of sampled chicken tenders.

[2]Sampling period following fresh and frozen storage of packaged chicken tenders.

[3] Storage period of chicken elapsed following harvest.

[a,b,c,d,e,f,g,h,i,j,k,l,m,n]Means lacking common superscripts differ ($P < 0.05$).

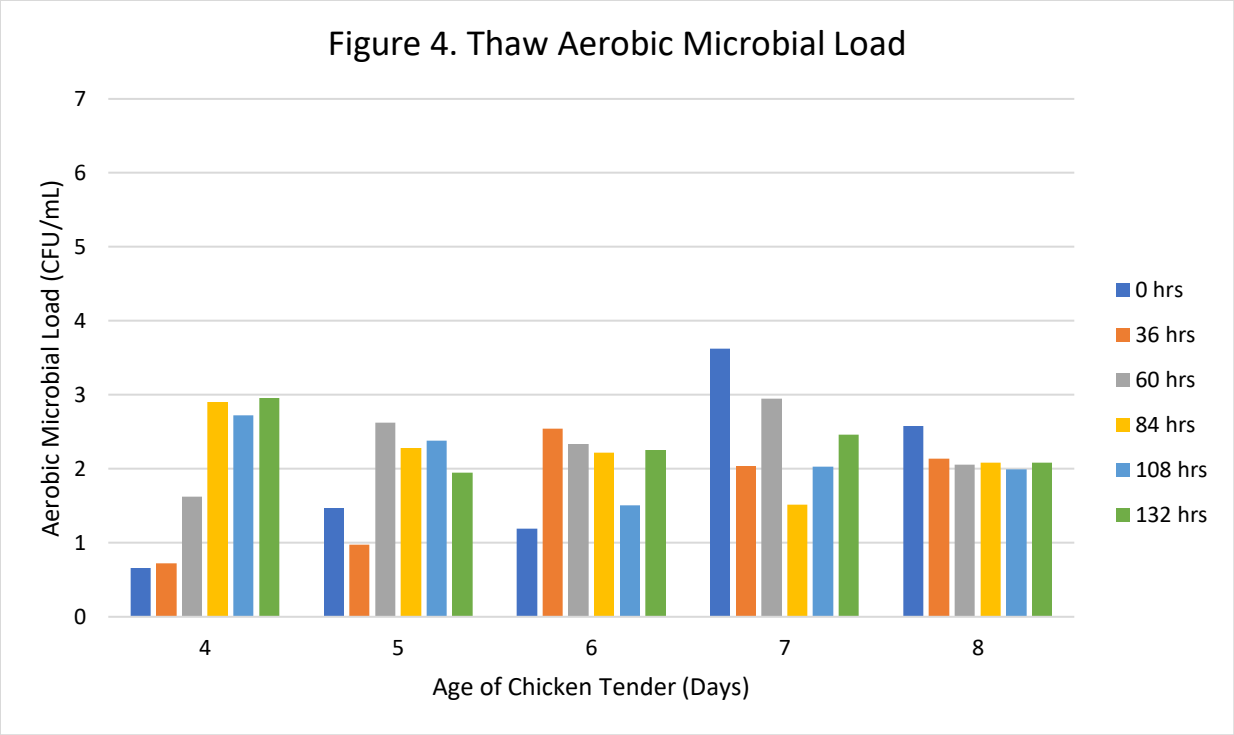


Figure 4. Thaw Aerobic Microbial Load
Aerobic Microbial Load (CFU/mL)
0
1
2
3
4
5
6
7
4
5
6
7
8
Age of Chicken Tender (Days)
0 hrs
36 hrs
60 hrs
84 hrs
108 hrs
132 hrs

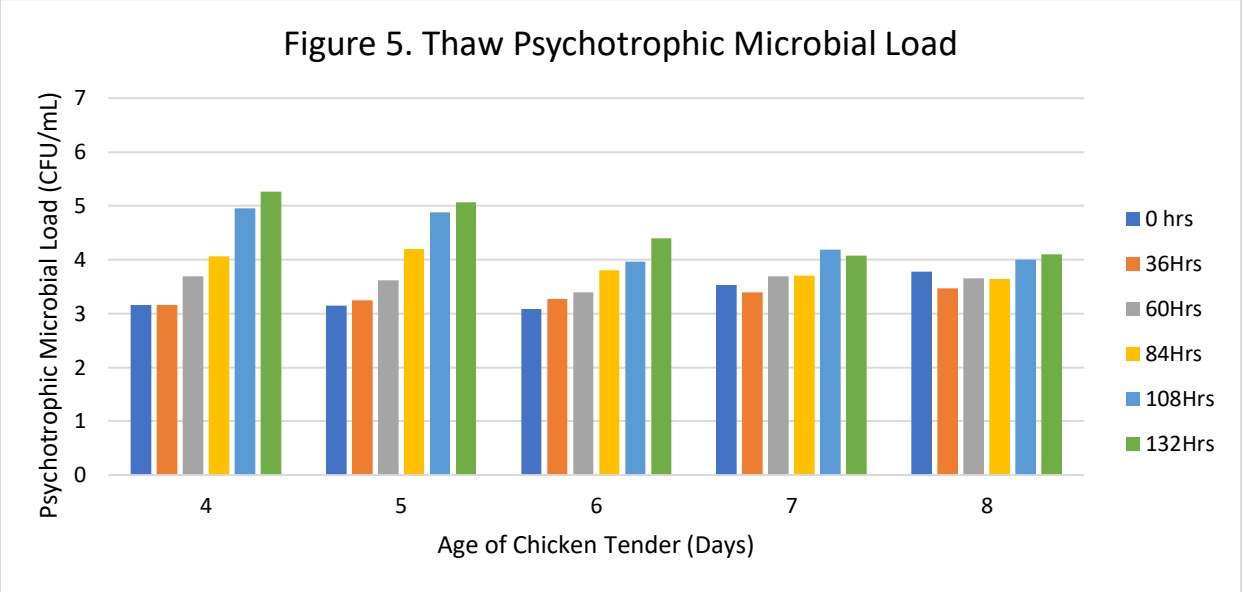


Figure 5. Thaw Psychotrophic Microbial Load
Psychotrophic Microbial Load (CFU/mL)
0
1
2
3
4
5
6
7
4
5
6
7
8
Age of Chicken Tender (Days)
0 hrs
36Hrs
60Hrs
84Hrs
108Hrs
132Hrs

**FIGURE 6.MARINATED CHICKEN PSYCHOTROPHIC GROWTH**

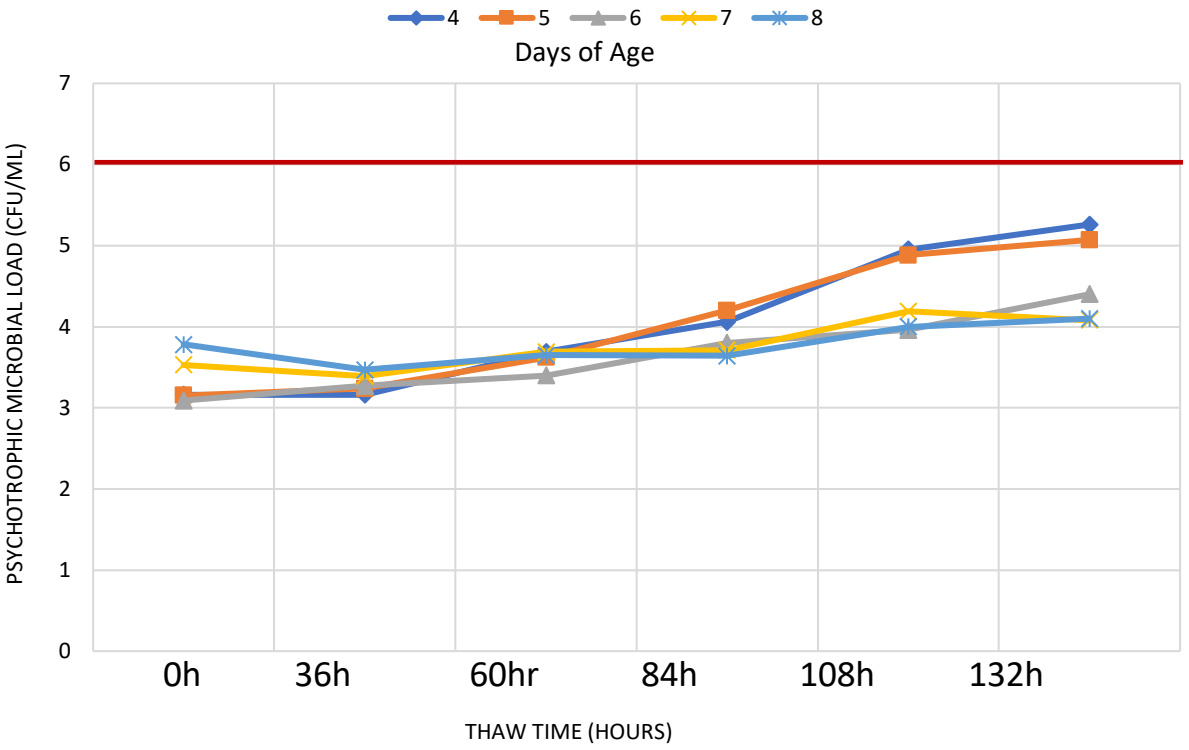


Figure 7. Fresh vs. Marinated Aerobic Microbial Load

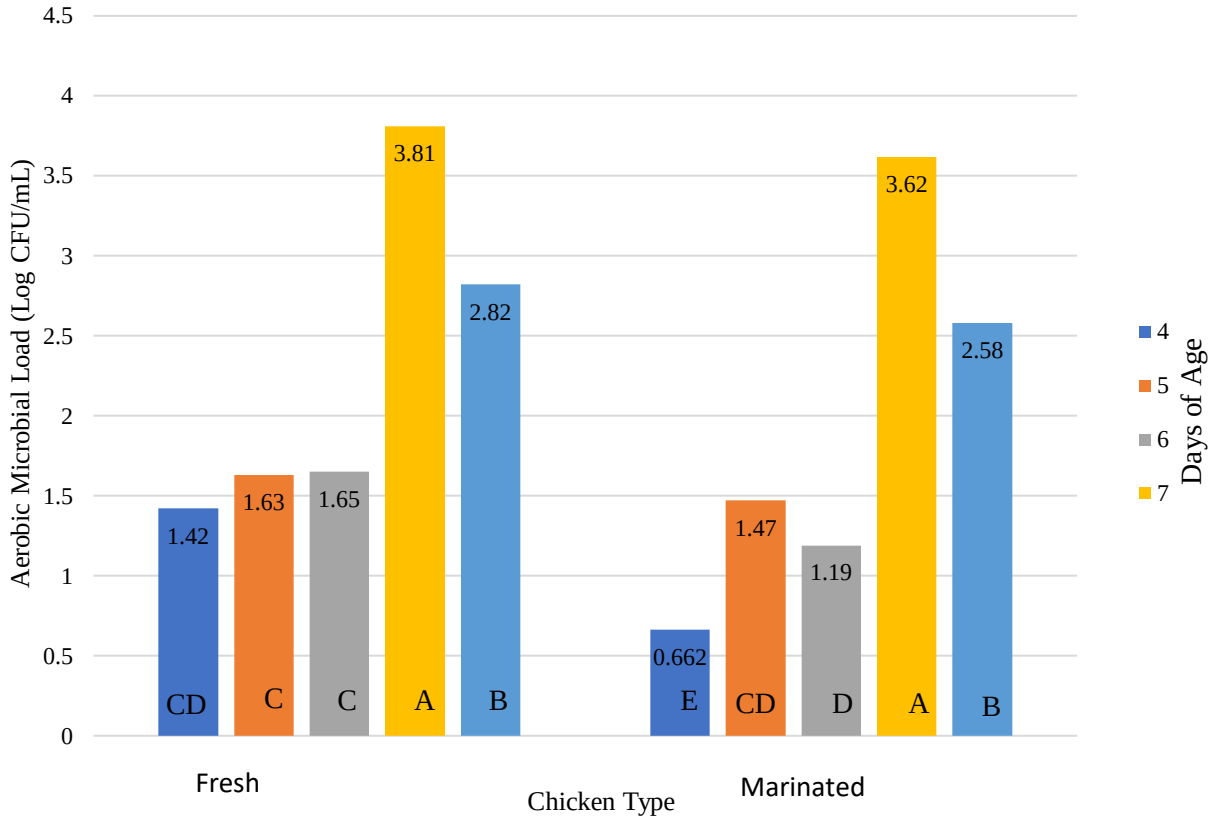


Figure. 8 Fresh vs. Marinated Psychotrophic Microbial Load

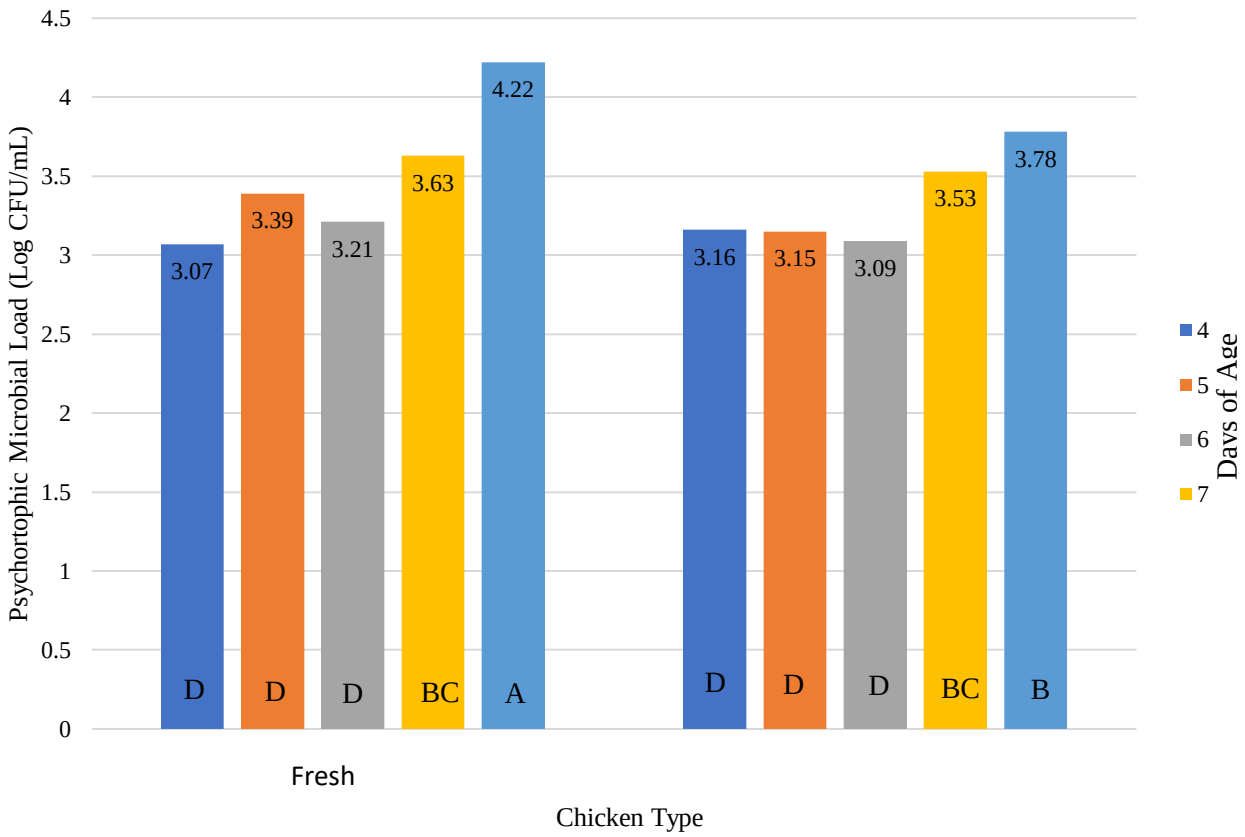


Figure. 9 Fresh vs. Marinated Lactic Acid Bacteria Microbial Load