EVALUATION OF CHILLING EFFICIENCY, MEAT TENDERNESS, AND MICROBIAL ANALYSIS OF BROILER CARCASSES USING SUB-ZERO SALINE SOLUTIONS

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Samira Viliani

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TITLE:	Evaluation of Chilling Efficiency, Meat Tenderness, and Microbial Analysis of Broiler Carcasses Using Sub-Zero Saline Solutions
AUTHOR:	Samira Viliani
DATE SUBMITTED:	September 2019
COMMITTEE CHAIR:	Iksoon Kang, Ph.D. Associate Professor of Animal Science
COMMITTEE MEMBER:	Darin Bennett, Ph. D. Assistant Professor of Animal Science
COMMITTEE MEMBER:	Amanda Lathrop, Ph.D. Associate Professor of Food Science & Nutrition

ABSTRACT

Evaluation of chilling efficiency, meat tenderness, and microbial analysis of broiler carcasses using sub-zero saline solutions

Samira Viliani

The poultry industry is seeking an advanced chilling system that can improve chilling efficiency, microbial safety, and water consumption without compromising meat quality. The objective of this study was to evaluate the effects of sub-zero saline chilling methods on chilling efficiency, breast fillet tenderness and microbial reduction of broiler carcasses. Following evisceration and rinsing, broiler carcasses were randomly assigned to one of three chilling solutions: 1) 0% salt or ice water control (0% NaCl/0.5^{o}C), 2) 3% salt (3% NaCl/-1.8^{o}C), and 3) 4% salt (4% NaCl/-2.41^{o}C) solutions. Broiler carcasses in sub-zero saline solutions reached the target internal temperature of < 4.4 ^{o}C in a faster rate than the 0% salt control, reducing the chilling time by 11% and 39 % for 3% NaCl/-1.8^{o}C and 4% NaCl/-2.41^{o}C solutions, respectively. There was no significant difference in breast fillet pH, regardless of chilling treatment ($P < 0.05$). However, the breast fillets from sub-zero saline solutions showed higher R-value and longer sarcomere length than those of control fillets ($P < 0.05$). Breast fillets excised from carcasses in 4% NaCl/2.41^{o}C were significantly tenderized more than the control fillets, with an intermediate tenderness observed for the fillets from 3% NaCl/-1.8^{o}C ($P < 0.05$). Before chilling, broiler carcasses contained mesophilic aerobic bacteria (MAB), *Escherichia coli* (*E. coli*), and total coliforms for 3.81, 0.78, and 1.86 log colony forming unit (CFU)/g, respectively. After chilling, the populations of *E. coli* and total coliforms were significantly reduced on the carcasses in 3% NaCl/-1.8^{o}C and 4% NaCl/-2.41^{o}C

compared to the control fillets ($P < 0.05$). There was no significant difference for MAB populations, regardless of treatment. Based on these results, chilling of broiler carcasses in 4% NaCl/-2.4 °C solution seems to be the best choice to improve chilling efficiency, meat tenderness, and microbial reduction compared to the control (0% NaCl/0.5ºC) and 3% NaCl/-1.8^{o}C solutions.

Keywords: Sub-zero saline chilling, Broiler carcass, Chilling efficiency, Meat tenderness, Microbial safety.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

Chapter 1

INTRODUCTION

1.1 Statement of Problem

The Food Safety and Inspection Service (FSIS), an agency of the United States Department of Agriculture (USDA), has implemented a final rule since 2001 that restricts the amount of water retained in poultry products caused by carcass washing and immersion chilling (Young and Smith, 2004; Northcutt et al., 2006). Chilling systems are constantly advancing in response to the poultry industry who are striving to improve chilling efficiency (shorter chilling time), reduce capital costs, improve microbial control, and adjust to new regulatory requirements without losing any quality of poultry products (Jeong et al., 2011a). The most challenging aspect in carcass chilling is to improve microbiological safety (i.e. reduce pathogenic microbes that impact food safety and shelf-life) and product quality (e.g. prevent tough broiler meat and pale, soft and exudative (PSE) turkey meat) (Petracci and Cavani, 2012; Demirok et al., 2013).

1.2 Purpose of Study

Poultry meat is widely consumed as an excellent protein source, with a relatively cheaper price than red meat (Botka-Petrak et al., 2005). The purpose of carcass chilling is to reduce the internal muscle temperature, and prevent microbial growth and muscle toughening from heat shortening (or rigor shortening) at 40 to 16 ^{o}C (Sams, 2001; Dunn et al., 1993a,b). Currently, the United States federal regulations require that the carcass temperature must reach 4.4°C or less within 4 to 8 h, depending on the post-slaughter

carcass weights (USDA, 2009). In broiler chilling, types of chilling methods impact the property of broiler breast fillet such as physicochemical, microbial, sensory, and culinary attributes (Mir et al., 2017).

In previous research, various saline contents and solution temperatures have been evaluated using 0% NaCl/0.5 °C to 8% NaCl/-5.08 °C and two sub-zero saline solutions (3% NaCl/-1.8 °C and 4% NaCl/-2.41 °C) were recognized as appropriate chilling solutions (Metheny et al., 2019). As a result, the purpose of this research was to investigate the chilling effects of the two sub-zero saline solutions on broiler carcasses for enhancing chilling efficiency, meat tenderness, and microbial reduction.

Chapter 2

LITERATURE REVIEW

2.1 Chilling of Broiler Carcasses

There are three common methods of poultry chilling: 1) water immersion chilling (WIC), 2) air chilling (AC), and 3) evaporative air chilling (EAC) or an integration of WIC and AC technology (Sams, 2001) (Figure 2.1, 2.3). In the United States (U.S.), WIC has been the primary method to cool poultry carcasses, as it is efficient and economic (Huezo et al., 2007). As shown in Figure 2.2, eviscerated birds are initially submerged in cold water (pre-chilling) that is flowing counter to the direction of carcass processing. They are then delivered to the next tank (post-chilling) filled with an ice and cold water mixture (Schmidt et al., 2008; Zhang et al., 2011; Rodrigues et al., 2015). Throughout the immersion chilling, carcasses were mechanically tumbled (4 rpm) in a chiller containing 50:50 ice and water (temperature range between 1-2°C) for 30 – 60 min, where they absorbed moisture up to 11.7% (Young and Smith, 2004).

Figure 2.1 Water immersion broiler chiller.
Source: (Beavers, 2015a).

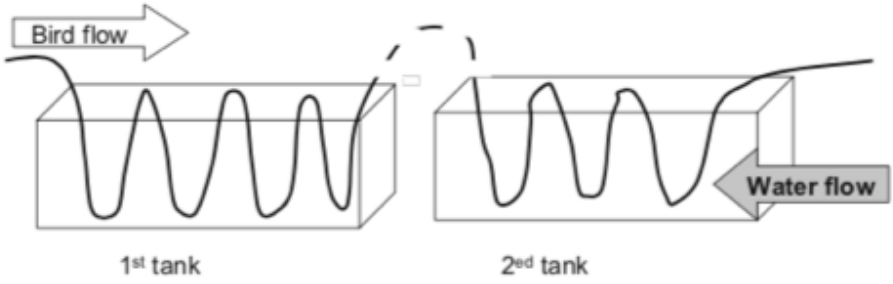

Figure 2.2 Diagram of a two-tank water immersion chilling system with counter flow. Source: (Barbut, 2010).

The most notable feature in the chilling tank is the corkscrew current that displaces poultry carcasses against the current of cooling water below 4°C (Carciofi and Laurindo, 2007). During the post-chilling stage, the carcasses are cooled in water at 1-2°C and capable of absorbing water (4 - 6%) via the skin and surrounding fat (Demirok et al., 2013). By the end of the chilling process, the temperature of carcasses should be reduced from 40 to 4°C in order to decrease microbial growth and maintain the product quality (Rodrigues et al., 2015). Currently, counter-current immersion chilling systems are used with a maximum water inlet temperature of 4°C in order to limit water absorption by the carcasses, length of dwell time in water, and degree of water agitation (James et al., 2006). Moreover, the FSIS of the USDA is concerned with unnecessary water absorption and requires additional water to be listed on the label (Zhang et al., 2011). Although WIC is predominant in the US, due to its superior chilling efficiency, as compared to other methods, the practice of WIC is currently facing challenges with cross-contamination, waste-water management, reshackling, postchill purge, and moisture uptake (Zhang et al., 2011; Rodrigues et al., 2015). Especially, the moisture uptake

causes poultry carcasses to exhibit higher drip loss, thaw loss, and cook loss traits (Huezo et al., 2007; Demirok et al., 2013). With regards to cook loss, Metheny et al. (2019) monitored the cooking yield (%) for broiler breast fillets derived from various chilling solutions (0, 1, 2, and 3% NaCl) and found no significant difference across treatments. It is theorized that the salt is trapped between in a space between skin and muscle, although the salt will penetrate the muscle over a long period of time.

Figure 2.3 Air or evaporative air (air/water) chilling technologies. Source: (Beavers, 2015b).

Air chilling method that are popular in Europe and South America has gained popularity in the U.S. due to reduced water usage, minimized wastewater discharge, and consumer preference (Figure 2.3). Currently, AC is getting support in U.S. after the changes of US federal regulations on carcass moisture retention (Huezo et al., 2007). The air chilling process involves cold air blasting into the abdominal cavity and exterior of thick portions (e.g. legs, breast) of poultry carcasses, thus enhancing the effectiveness and consistency of carcass chilling (Jeong et al., 2011b). In fact, the average AC system

operates with an air blast near 0°C in a separate room or tunnel for 90-150 min until the carcass temperature meets regulatory requirements (Rodrigues et al., 2015). The primary advantages of air-chilled carcasses are no moisture uptake, tenderized meat, better marinade pick up, and overall consumer acceptance (James et al., 2006; Carciofi and Laurindo, 2007; Zhang et al., 2011; Demirok et al., 2013). However, the lack of moisture uptake and moisture loss can induce a negative yield for the carcasses (Demirok et al., 2013). In comparison to WC poultry carcasses, AC carcasses appear drier and lose weight by 0.8 to 2.5% (Huezo et al., 2007) (Figure 2.4) (Jeong et al., 2011b). Sometimes, the weight loss associated with air-chilled carcasses can even escalate to 3%, depending on carcass capacity and chilling system requirements, which often causes surface dehydration and discoloration (James et al., 2006; Demirok et al., 2013).

Figure 2.4 Visual appearance of broiler carcasses chilled in water, air, and abnormal air conditions (Kang, Not published)

Nonetheless, there are conflicting research results and controversial debates about which chilling system yields less microbial populations. Several studies have observed reduction of microbial load on broiler carcasses in WIC due to washing effect and antimicrobial agents (e.g. organic acids, chlorine) (Bilgili et al., 2002; Demirok et al.,

2013; Northcutt et al., 2003, 2008). Conversely, other studies indicate that AC reduces bacterial populations and cross-contamination because carcasses are arranged and hung separately on a line, regardless of a water spray in the AC system (James et al., 2006; Demirok et al., 2013). Several studies have shown that AC products have lower aerobic plate counts than WIC products during chilling, thus creating an environment where bacterial populations cannot proliferate (Berrang et al., 2008; Carroll and Alvarado, 2008; Demirok et al., 2013). Ultimately, the difference in microbial populations between the two chilling methods depend on various factors such as prechill carcass microflora, type of chiller equipment, antimicrobial levels, and sampling techniques (Zhang et al., 2011).

2.2 Saline Solution Chilling

Many antimicrobial agents are utilized to decontaminate fresh meat or poultry products during processing. Most of them are not regarded as generally recognized as safe (GRAS) but are used to prevent bacterial contamination and growth on carcasses (Simpson and Sofos, 2009). In the food industry, sodium chloride (NaCl) is a highly utilized and most effective preservative (Alvarado and McKee, 2007; Shin et al., 2012). Salt and other antimicrobial interventions must gain FSIS approval to decontaminate carcasses by satisfying the requirements of GRAS. (Simpson and Sofos, 2009). Osmotic treatment involves full immersion of a meat sample in an aqueous solution containing at least one of the osmotic agents such as salts, sugars, phosphates, and acids (Schmidt et al., 2008). Salt is capable of being dissolved in water, thus increasing the ionic strength of the water (Alvarado and McKee, 2007). Under these conditions, salt provides an intervention strategy for meat with minimal changes in visual and organoleptic property. (Simpson and Sofos, 2009).

The primary outcome of osmotic treatment with salt is water and salt exchanges, one-way or counter-current flow based on osmotic solution concentration, which causes meat hydration or dehydration (Schmidt et al., 2008). The ionic strength of poultry tissue fluid is lower than the brine solutions having 0.8% NaCl or more, leading to osmosis and brine solution uptake by the meat until equilibrium is achieved (Alvarado and McKee, 2007). Therefore, the goal is to immerse the meat portions in moderately low concentrated brine solutions to improve meat sensory properties such as juiciness and flavor (Schmidt et al., 2008). The addition of salt in muscle tissue can increase water absorption coupled with an expansion in tissue volume (Mohamed-Ali and Heath, 1991). A post rigor mortis muscle became swollen when it was immersed in a large volume of NaCl solution (1M), specifically an 80% surge in tissue expansion (Schmidt et al., 2008). Carcass chilling in sub-zero brine chilling solutions can improve chilling efficiency by lowering the solution temperature below the precedent commercial standard of 1-4°C (Metheny et al., 2019). The incorporation of sodium chloride to water reduces the freezing point of the solution, as compared to pure water. In fact, the freezing temperature depression is negatively correlated with the salt concentration; therefore, the higher concentration of salt in the brine, the lower the solution temperature before freezing (Metheny et al., 2019). Moreover, sub-zero brine chilling has the potential to improve the tenderness of broiler muscle. Previously, broiler carcasses immersed in 5% NaCl solution at -1°C for 4 hours exhibited an increase in muscle tenderness (Hoey et al., 1983) by increasing water holding capacity and lowering muscle shrinkage (Janky et al., 1978).

2.3 Broiler Chilling Rates

Currently, poultry processors are seeking a new chilling method that can reduce chilling time, weight loss, energy consumption, and capital costs without compromising product quality and safety. The poultry chilling regulations in the United States require carcasses to be chilled to 4.4°C or lower in 4, 6, or 8 h for carcasses weighing less than 4, 4-8, or over 8 pounds, respectively (James et al., 2006). Jeong et al. (2011b) evaluated the efficiency of three chilling methods (WIC, AC, and EAC) by monitoring the temperature reduction of broiler carcasses from the initial (39.9°C) to the end chilling (4°C) and reported the average chilling times of 55, 155, and 120 min, respectively (Figure 2.5). James et al. (2006) found that the time required to chill a 1 kg carcass to 4°C was 25 min at -5 to -18°C, but was 55 min at 0°C.

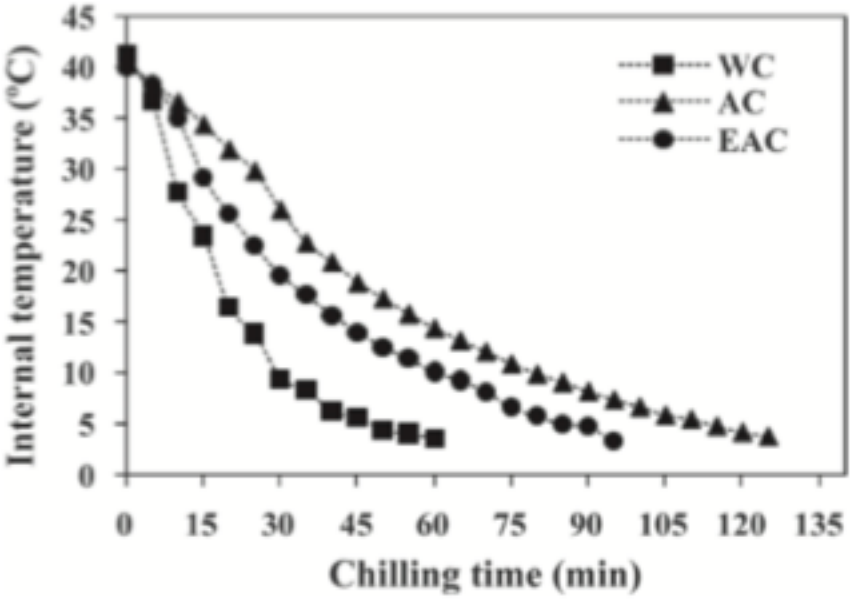

Figure 2.5 Temperature change profiles of broiler breast fillets during water chilling (WC; circle), air chilling (AC; triangle), and evaporative air chilling (EAC; square). Source: (Jeong et al., 2011a).

Even though faster chilling was achieved in WIC than AC, the WIC holds the higher operating costs and water/sewer costs (Huezo et al., 2007; Demirok et al., 2013). In general, chilling rates in immersion chill systems are influenced by the types of cooling media, temperatures of the media, sizes of carcasses, and conditions of wrapping or unwrapping (James et al., 2006). Bekhit et al. (2014) reported that the chilling rates of unwrapped carcasses were faster than those of wrapped carcasses, which extended the chilling time double. Wrapping of pre-rigor muscles with polyethylene film has been used to inhibit the muscles from shortening during rigor development (Bekhit et al., 2014). More specifically, muscle contraction is halted by preventing the diametrical muscle contraction via the compression force generated from the elastic film on the meat. Aside from WIC and AC, EAC presented an intermediate chilling rate because the water

spray reduced the evaporative heat loss in carcasses compared to air chilling (James et al., 2006; Jeong et al., 2011a).

2.4 pH

Muscle pH directly impacts the meat quality such as tenderness, water-holding capacity, color, juiciness and shelf life (Mir et al., 2017). Under normal processing conditions, the pH of fresh muscle falls from 7.0 to 5.3-5.8 (Savell et al., 2004). There are several methods to measure the final pH of broiler breast meat. One of them is to calculate pH as total glycogen concentration in the breast muscle at the time of slaughter deducted by the rate of glycogen conversion into lactic acid post-slaughter. However, obtaining the pre-slaughter glycogen concentration in living muscle is challenging and likely producing inaccurate results due to muscle stimulation and glycogen conversion into lactate during the sampling process (Van Laack et al., 2000). Low levels of glycogen in the live animal indicates minimal lactate generation and an ultimate pH greater than 5.5 (Dransfield, 1994). An alternative method is measuring the glycolytic potential, which is the sum of carbohydrates (that might convert to lactate) plus the amount of present lactate (Van Laack et al., 2000). However, Van Laack et al. (2000) analyzed that post-mortem muscle samples undergoing anaerobic respiration yielded constant glycolytic potential (97 μmol/g), whereas pale muscle samples showed no different glycolytic potential (101 μmol/g); a significant low pH with glycolytic potential correlation coefficient (-0.32) contradicts the alternative method of pH measurement since the differences in pH cannot explain the variability in glycolytic potential.

Regardless of pH measurement method, low pH and high muscle temperature results in PSE meat traits such as lower water holding capacity, less brine pickup, and poorer fillet color (Alvarado and McKee, 2007). As the pH declines, it descends near the isoelectric point, where all of the negative and positive charged amino acid side chains are equivalent, inducing the maximum muscle contraction (Savell et al., 2004). The thick and thin filaments are held densely as a result of this contraction, which does not permit water to enter any available spaces, thus greatly lowering the water holding capacity. The degree of protein denaturation and visual appearance of meat are highly dependent on post-mortem temperature and pH; the relationship between post-mortem temperature and pH influences the amount of light reflected from the interior and exterior of the meat surface as light scattering is directly proportional to the extent of protein denaturation (Mir et al., 2017). Light scattering is amplified by meat lightness (L*) in a manner inverse to scattering caused by heme pigment concentration. In particular, low pH causes the proteins in the muscle to disperse, resulting in light reflecting differently at the surface and emits light color. The exudate or water on the meat surface, common with PSE meat, appears glossy and reflects more light, hence affecting color measurements (Kim et al., 2014). Muscles at pH greater than or equal to 6.0 indicate minimal protein denaturation, low light scattering, and translucent appearance. Conversely, muscles at pH less than or equal to 6.0 undergo greater protein denaturation, causing increased light scattering and opaqueness (Mir et al., 2017).

2.5 Tenderness

Meat texture is considered one of the most important quality characteristics for consumer acceptance and satisfaction when consuming poultry meat products. During

processing, the rate and degree of chemical and physical changes in the muscle can affect tenderness. The starting point for changing meat texture is the slaughter, where exsanguination halts the bird's blood circulation and obstructs the flow of oxygen-bound blood cells or nutrients to the muscles (Mir et al., 2017). Once the muscles have no more energy reserves to sustain glycolysis, they undergo rigor mortis development. Any deviation in the conventional conversion of muscle to meat will impact its tenderness. The rate of post-mortem energy metabolism of the muscle or consumption of energy precursors/substrates such as ATP and creatine phosphate, is a key determinant of post-mortem sarcomere shortening and proteolysis of muscle (Lonergan et al., 2010; Kim et al., 2014). In fact, it is postulated that variation in tenderness during the rigor mortis is the result of proteolysis and amount of actin/myosin cross-bridges (Santos et al., 2004). As the muscle transitions from the resting (or weak) to contractile (or strong) state, it is succeeded by a steady weakening of the contractile muscle state; thus, imparting a significant role in meat texture (Santos et al., 2004). In general, meat tenderness can be affected by the status of muscle shortening, amount of connective tissue, and level of post-mortem proteolysis (Kim et al., 2014).

2.5.1 Accumulation of Connective Tissue

The three most significant factors to poultry meat tenderness are the maturity of connective tissues, contractile state of myofibrillar proteins, and proteolytic enzyme activity. With regards to connective tissue maturity, collagen cross-linking increases with age and results in tougher meat in older birds (Fletcher, 2002). However, the modern broiler industry has tackled the age-related toughness. In particular, the chicken market has made it more profitable and efficient by reducing the market age of broilers to less

than 7-8 weeks (Fletcher, 2002). While it remains unclear whether the total amount of muscle collagen is influenced by age or not, it resists to heat and salt solubility with age increases (Mir et al., 2017). The variation in collagen strength is determined by the change in total collagen fibers and the angle in which these fibers align with muscle fibers (Dransfield, 1994). Based on these parameters, tension created in the connective tissue can contribute more meat toughness.

2.6 Rigor Mortis Development

Currently, there is a high demand for cut up, deboned, and further processed products, which places more pressure on processing plants to produce these products by deboning carcasses as quickly as possible (Fletcher, 2002). When carcasses are cut up into parts before the completion of rigor mortis, the muscle will contract and shorten. The development of rigor mortis is contingent on the rate of glycogen depletion and the metabolic activity of muscle tissues (Grashorn, 2010). For a living muscle, high levels of ATP are sustained by oxidizing organic compounds and aerobic respiration (de Fremery and Pool, 1960). After animal exsanguination, the muscle is still extensible because there is available ATP that can bind with magnesium (Mg^{2+}), which aids in disrupting the actomyosin cross-bridges and the muscle can relax (Savell et al., 2004; Lonergan et al., 2010). This pre-rigor, or delay stage in the course of muscle toughness, can sustain anaerobic respiration without oxygen because creatine phosphate (stores for phosphate bond energy) proceeds to regenerate available ATP from ADP (Figure 2.6) (de Fremery and Pool, 1960; Savell et al., 2004). There is no pH drop during these first few minutes to 30 min post-mortem but lactic acid is beginning to accumulate due to anaerobic respiration (Lonergan et al., 2010; Guerrero-Legarreta and Hui, 2010). Once the reserves

of glycogen and creatine phosphate are depleted, the phosphorylation of ADP into ATP is hindered, leading to ATP decline rapidly (de Fremery and Pool, 1960; Lonergan et al., 2010). With little to no ATP available to disrupt actin and myosin bonds, the contraction-relaxation cycle ceases and muscular extensibility steadily recedes (Savell et al., 2004; Guerrero-Legarreta and Hui, 2010). This stage is characterized as the rapid phase because the development of rigor bonds progress at a high-speed rate and actomyosin cross bridges can cause myofibril shortening (Lonergan et al., 2010). Lastly, the post-rigor phase is defined as the complete depletion of ATP reserves and large buildup of lactic acid, triggering the pH of the meat (initially at 7.0) to decline to final levels of 5.5-5.7 (de Fremery and Pool, 1960; Guerrero-Legarreta and Hui, 2010). A muscle tissue that has completed rigor mortis has a permanently rigid structure.

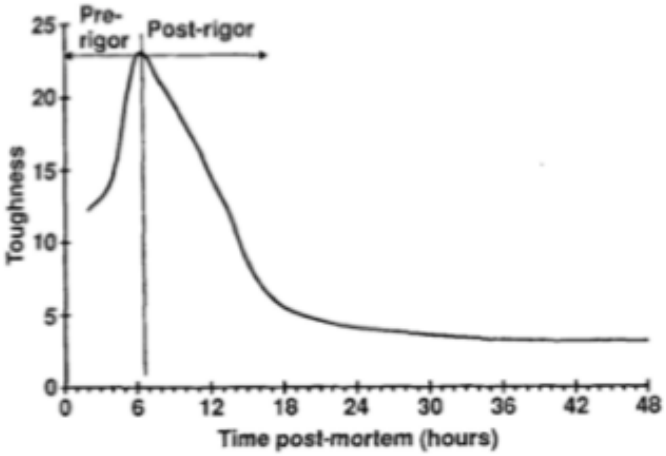

Figure 2.6 Ordinary course of muscle toughness in chicken breast (*Pectoralis major*) muscle. Source: (Schreurs, 2000).

2.6.1 Post-Mortem Temperature

The post-mortem temperature of meat has an influential relationship with the progression of rigor mortis in assessing the quality of poultry meat (Berri, 2000). Unlike the red meat species (e.g. beef, lamb, and pork), the rigor mortis process in broiler muscle is notably rapid and complete within 2-3 h post-slaughter (Dunn et al., 2000; Petracci et al., 2009). When measuring the temperature on eviscerated carcasses, the breast muscle takes the most time to be chilled, hence an accurate measurement can be taken in the center of the thickest muscle or the 'breast geometric center' (Thomson et al., 1974; James et al., 2006; Rodrigues et al., 2015). During poultry processing in commercial plants, broiler carcasses can be exposed to the temperatures as low as 0°C during chilling and their initial muscle pH can vary from 6.90 to 5.90 (Dunn et al., 2000). Furthermore, Dunn et al. (2000) found that variability of muscle pH and temperature can negatively impact on meat tenderness since both parameters impact the type and amount of muscle shortening. One recommendation for preventing meat defects is adapting the chilling rate (slow or rapid) to the intrinsic properties of the meat, but this would require processors to create a prompt on-line measurement of rigor mortis development (Berri, 2000; Grashorn, 2010). There are few targeted studies on post-mortem temperature, pH decline, protein properties, and broiler meat qualities. Sams (2001) reported that the combined effects of elevated post-mortem temperature (40°C) and escalated glycolysis rate resulted in pale surfaces and low water-holding capacity of broiler muscle

One of the most persistent challenges for the meat industry is the occurrence of PSE meat linked with intensive selection for increased muscling (Petracci and Cavani, 2012). There is extensive research examining the correlation between high post-mortem

temperature and low pH in generating pale and exudative characteristics in pork, but there is a rising number of cases on PSE meat in poultry (Sams, 2001; James et al., 2006; Petracci et al., 2009). Besides genetic factors, heat stress during growing phase or preslaughter stage is a major environmental contributor of PSE-like broiler meat; fast-growing heavy birds have shown more sensitivity to heat stress, resulting in higher metabolic heat production, elevated body temperature, and mortality (Petracci and Cavani, 2012).

It is estimated that 5-40% of poultry breast produced in the U.S. show PSE traits, with higher rates in the summer season, translating to roughly 200 million dollars in annual revenue losses for the poultry industry (Grashorn, 2010; Desai et al., 2016). The postmortem metabolic pathways in poultry are based on acute heat stress. Higher levels of stress will intensify superoxide free radical production in broiler skeletal muscle, which is mainly responsible for changes in meat quality, alterations in tissue structure, and oxidative damage (Petracci and Cavani, 2012). As shown in Figure 2.7, PSE-like poultry meat is depicted as containing light color, flaccid texture, low water-holding capacity, and reduced cooking yield (Zhu et al., 2011; Petracci and Cavani, 2012), however there is limited information on how temperature affects the meat quality during the early stages of rigor mortis. The protein quality loss was the result of muscular acidification plus high muscle temperature in the early post-mortem stage (within 30 min after death) (Petracci and Cavani, 2012). With regards to temperature, Zhu et al. (2011) found that broiler breast muscles exposed to temperatures 0°C and 40°C produced severe shortening compared to breast muscles exposed to 10-30°C, therefore impacting cooked meat quality. Interestingly, poultry muscle has a pH less than 6.0 while the carcass

temperature remains above 37°C, yet not all poultry meat exhibits PSE-like characteristics (Van Laack and Lane, 2000).

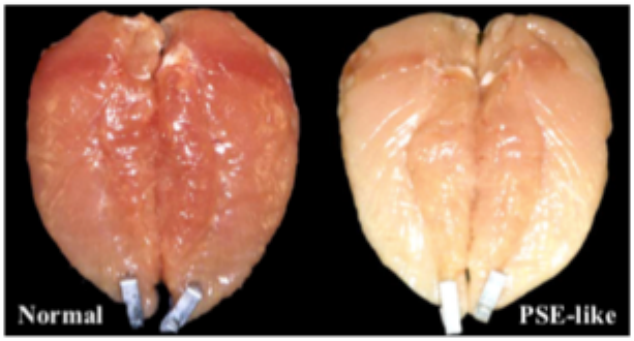

Figure 2.7 Comparison of normal breast meat and pale, soft, and exudative (PSE)-like broiler breast meat. Source: (Petracci & Cavani, 2012).

2.6.2 Rigor Shortening

The meat industry has expedited carcass processing in order to increase production yields, however this short processing time does not account for carcasses transitioning into the chilling system prior to the completion of rigor mortis (Grashorn, 2010). During the early stage of chilling, poultry muscle presents rigor shortening when the muscle temperature is 10°C or higher and pH is 6.20 or less (Dunn et al., 2000). Rigor shortening, also known as hot shortening, is largely influenced by early post-mortem temperature and pH of the muscle (Lonergan et al., 2010). Papinaho and Fletcher (1996) narrowed the chilling temperature range for rigor shortening and cold shortening between 37 to 4°C. Exposure of pre-rigor muscle to the temperatures of 0-10°C results in muscle shrink by 50% of its fully-extended length (Lonergan et al., 2010). The muscles that undergo 40-50% shortening reach maximum toughness since the length of sarcomere is approximately the same length as thick filaments. Once the meat is cooked, the crosslinks

of thick and thin filaments create a tough continuum with filaments from adjoining sarcomeres (Dransfield, 1994). As the temperature range shifts to 15-20°C, the post-mortem muscle will experience the lowest level of rigor shortening (10%) since the pre-rigor stage can gradually develop into rigor at this range. Pre-rigor muscles chilled in 20-40°C can experience considerable rigor shortening, as much as 30% of the initial sarcomere lengths (Lonergan et al., 2010). Isolated *Pectoralis major* strips of broilers underwent extensive rigor shortening at 40°C as the muscle pH descended to 6.2 (Dunn et al., 1995). Although the chilling temperature range 15-20°C presents ideal meat texture for poultry processors, it would be problematic to store pre-rigor meat at this range considering microbiological safety.

An early study validated that the chemical reaction, most closely correlated with the onset of rigor mortis, is the reduction of adenosine triphosphate (ATP), glycogen and muscle pH (de Fremery and Pool, 1960). Given this description, the execution of rigor mortis development can be interpreted as the third stage of post-rigor, where the muscle extensibility has reached a constant minimal level (Papinaho and Fletcher, 1996).

De Fremery and Pool (1960) analyzed the rate of ATP consumption by chilling hot-boned chicken breast fillets in polyethylene bags at five different temperatures (0, 10, 20, 30, 40°C) and compared four sets of temperatures (0 vs. 10°C, 10 vs. 20°C, 20 vs. 30°C, and 30 vs. 40°C). They reported that breast fillets stored at 0°C showed a marked, continuous decline in ATP concentration at 4 h compared to 10°C, and the ATP concentrations gradually decreased overtime at the same rate for 10 and 20°C as well as 20 and 30°C. Breast fillets stored at 40°C exhibited a sharp and continuous drop after 2 h post-mortem as compared to fillets stored at 30°C (de Fremery and Pool, 1960). For

broilers, Papinaho and Fletcher (1996) reported that hot-boned breast muscle demonstrated higher degrees of rigor shortening in the anterior end compared to the posterior end; rigor shortening may be influenced by intramuscular location based on sarcomere length measurements in the *Pectoralis major* and *Pectoralis minor* muscles.

2.6.3 Cold Shortening

Unlike rigor shortening, cold shortening is defined as a rapid muscle shortening when the muscle is exposed to the cold temperatures at 14-19°C or less before the onset of rigor mortis (Demby and Cunningham, 1980; Savell et al., 2005; James et al., 2006; Kim et al., 2014). Chilling a pre-rigor muscle at the temperature range 0 to 15°C compromises the functionality of the sarcoplasmic reticulum, and this structure leaks substantial cellular calcium concentrations into the sarcoplasm (Kim et al., 2014). The degree of muscle shortening coincides with the amount of calcium ions leaked into the sarcoplasm (Woods and Richards, 1974). Besides the copious calcium, there is still ATP in the muscle that fuels contractions followed by diminished tenderness. If the muscle was chilled at 1 to 2°C, the sarcoplasmic reticulum would be completely dysfunctional and undergoes similar reactions.

Cold shortening can occur in early deboned broiler breast muscles, especially when they are not under normal processing conditions or no longer attached to the skeletal framework of the carcass (Papinaho and Fletcher, 1996). Dunn et al. (1995) extracted *Pectoralis major* chicken strips and observed cold shortening when the muscle was exposed to 0°C and muscle pH was above 6.7. In an follow-up study, strips of *Pectoralis major* muscle showed cold shortening when the muscle pH is greater than or

equal to 6.70 and held at temperatures 5°C or less (Dunn et al., 2000). Early studies in 1960s delineated the direct relationship of cold shortening and sarcomere length to fiber diameter and toughness. Theoretically, the more the sarcomere is contracted, the more the fiber becomes bigger in diameter (Savell et al., 2005). Once the meats are cooked, the meats with bigger fiber diameter are tougher than those with smaller fiber diameter. Another key factor in determining the degree of cold shortening is muscle type. Poultry breast muscles are predominantly white muscle fibers, which are less prone to cold shortening than red muscle fibers (James et al., 2006). On the contrary, Savell et al. (2004; 2005) countered that white muscle fibers have larger glycogen stores, which would leave the fibers more susceptible to a severe drop in pH and quicker development of rigor mortis as compared to red fibers (2004; 2005). Given these points, the association between muscle fiber type and development of cold shortening remains a debated subject with conflicting theories and data.

Even though there is no guaranteed method to prevent rigor mortis and shortening of sarcomeres, there are some factors that can lessen the extent and toughening effects of cold shortening before, during, and after slaughter. Before slaughter, the factors that can influence toughening include carcass weight, fat thickness, and composition (Savell et al., 2005). Throughout the process of slaughter, toughness can be impacted by the methods of carcass slaughter, electrical stimulation, and suspension. After slaughter, two factors that impact carcass toughening are the time elapsed until carcasses are moved into cooler and cooler temperature. When broiler carcasses were stored at 3°C for different times (0, 2, 4, 8, 12 and 24 h) to examine post mortem toughness, Santos et al. (2004) found that

shear force dramatically declined in the 2 h treatment and continuous drop as the time progressed during storage.

One of many sarcomere-shortening conditions is thaw rigor, a type of rigor mortis that progresses when pre-rigor muscle was frozen in extremely cold temperature then thawed (Savell et al., 2004). During thawing the muscle, calcium is quickly discharged into the sarcoplasm, which caused a severe contraction followed by shortening of 60-80% of the initial muscle length, large emissions of juices, and extreme toughening (Savell et al., 2004). De Fremery and Pool (1960) excised chicken (*Pectoralis major*) muscles rapidly after slaughter and inserted them into polyethylene bags where one muscle sample was frozen immediately in a dry ice ethanol bath and stored at -23°C while the other muscle was cooled in tap water (13-14°C). All samples were routinely examined for ATP and glycogen levels during frozen then thawed state. These results indicated that ATP and glycogen levels disappeared more rapidly in the frozen then thawed muscle as compared to the unfrozen muscle. The immediate breakdown of glycogen after thawing the frozen samples was linked with pH decline, therefore the toughening effect of pre-rigor freezing is due to the accelerated onset of rigor mortis (de Fremery and Pool, 1960).

2.6.4 Proteolysis Enzymes

Through the aging process, myofibrillar proteins are degraded by endogenous proteolytic enzymes in the muscle, generating more tenderness in the meat (Kim et al., 2014). Generally, meat is tenderized as post-mortem enzymatic activity increased and breaking down myofibrillar proteins such as titin, nebulin, troponin-T, desmin, and

filamin; in particular, the most important breakdown product is derived from troponin-T (Xiong et al., 2012; Hui, 2006).

Currently, there are four major proteolytic enzyme systems identified in skeletal muscles that induce protein degradation into free amino acids or small peptides. Two (lysosomal and proteosomal) out of the four result in more generalized proteolysis whereas the other two (calpain and caspase) result in a more limited yet specified proteolysis during the early post mortem stages (Xiong et al., 2012). The synergistic action of three proteolytic systems-cathepsins, proteasome, and calpain-have been researched by many scientists, and a serious of studies demonstrate that the calcium-dependent system is vital for meat tenderization (Lee et al., 2008).

Generally, there is established research on the importance of calpains in post-mortem degradation and meat tenderness. The calpain enzyme system consists of numerous isoforms of the enzyme calpain and an endogenous inhibitor known as calpastatin (Lonergan et al., 2010). Of the many isoforms, μ-calpain and m-calpain cleave similar myofibrillar proteins that are also broken down during post-mortem aging. Even though these isoforms and calpastatin require the presence of calcium for their activity, μ-calpain and m-calpain are capable of autolysis, in living cells and post-mortem muscle, when incubated with calcium (Lonergan et al., 2010). Both μ-calpain and m-calpain can experience slower rates of activity towards myofibrillar protein substrates pH and ionic strength values similar to those in post-mortem muscle. In fact, an accelerated drop of early post-mortem pH has shown to support an expedited autolysis rate and activation of μ-calpain. It is postulated that endogenous calpains serve in targeted proteolysis of cytoskeletal proteins (e.g. titin and nebulin) as well as intermediate filaments (e.g.

desmin) to activate myofibrillar protein degradation (Lonergan et al., 2010). The activities of proteasome and caspase proteinase systems in post-mortem muscle are still being investigated, and the changes made to the muscular structure and their influences on meat quality are not definitive.

Overall, proteolysis consists of three consecutive stages: (1) action of calpains and cathepsins on major myofibrillar proteins that generate protein fragments and intermediate size polypeptides; (2) the resulting fragments and polypeptides are further hydrolyzed into small peptides by di-and tripeptidyl peptidases; and (3) dipeptidases, aminopeptidases, and carboxypeptidases are the last proteolytic enzymes that act on previous polypeptides and peptides to produce free amino acids (Hui, 2006) (Figure 2.8). The progression of proteolysis varies based on the processing conditions, muscle type, and the amount of endogenous proteolytic enzymes (Xiong et al., 2012).

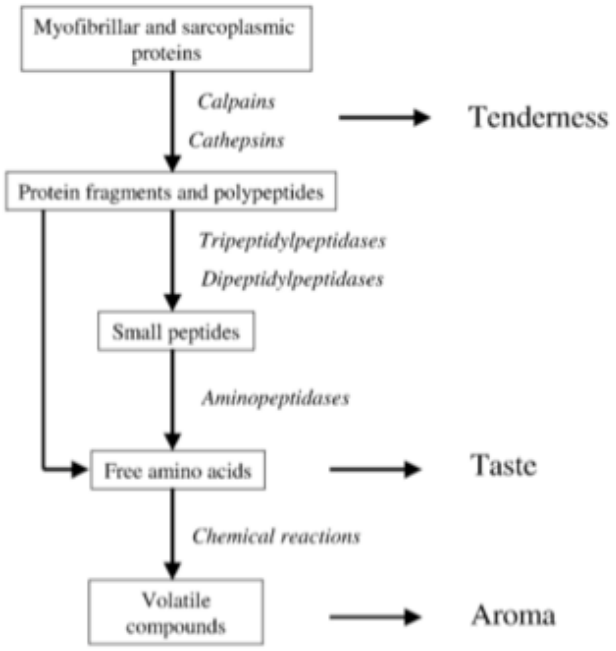

Figure 2.8 General scheme of proteolysis during the processing of meat and meat products. Source: (Toldrá, 2006).

2.7 Product Quality

2.7.1 Water-Holding Capacity

Water-holding capacity (WHC) describes the amount of water that is retained by meat (Kim et al., 2014). An increase in the water content of muscles produces enhanced tenderness, juiciness, and appearance; all of these attributes significantly improve the quality and economical value of meat. WHC is one of the most critical properties of raw meat and the outcome of interacting components such as the rate of pH decline during muscle to meat transition, sarcomere length and shortening during rigor mortis, and other factors such as ionic strength, pre-rigor myosin denaturation, osmotic pressure, and proteolysis (Kim et al., 2014; Mir et al., 2017).

After animal death, the shortage of oxygen in muscle triggers the accumulation of lactic acid, resulting in a pH decline. A low pH leads to the following cascade of events: protein denaturation, lack of protein solubility, and overall depletion of reactive groups for water binding on muscle proteins (Mir et al., 2017). In other words, the decline of reactive groups is caused by the muscle pH reaching the isoelectric point, where positive and negative charges on the proteins' reactive groups are equally attracted. If all reactive groups are neutrally charged, this leaves little to no electrical charge available to react with the charged groups of water; therefore, impeding the proteins' ability to bind with water (Mir et al., 2017). Van Laack et al. (2000) measured lower drip losses in pale broiler breast meat than PSE pork, likely due to the final pH of broiler meat (5.7) being further away from the isoelectric point of myosin (5.3) than the pH of PSE pork (5.4). The differences in final pH between pale broiler meat and PSE pork provide insight on impaired WHC and drip loss.

Besides the effects of low pH, the lack of energy generates a buildup of actomyosin complexes, causing insufficient space between myofibrillar proteins for water, thus a decline in WHC. As rigor mortis advances, divalent cations (e.g. $Mg^{2+}$and Ca^{2+}) in the sarcoplasm neutralize the negatively charged reactive groups on adjacent protein chains, thus decreasing the electrostatic repulsion between them (Mir et al., 2017). The reduced electrostatic repulsion between reactive groups also depletes available space for water to be held intramuscularly, which increases the amount of water released to the extracellular space. In fact, salts act by unfolding myofibrillar proteins and solubilizing them in saltwater solutions (Pearce et al., 2011). The unfolding of myofibrillar proteins is the result of electrostatic repulsion of chloride ions, thus the

charged binding sites are exposed. Together with exposing more charged sites for water binding, the electrostatic repulsion will expand the space between thin and thick filaments (Pearce et al., 2011). Consequently, the size of the space between filaments coincides with the amount of water retained by the muscle. Altogether, any variable factor that influences the spacing between thin and thick filaments, or the proteins' capability to bind with water, can influence WHC properties of meat.

As shown in Figure 2.9, the most essential myofibrillar proteins associated with water binding and meat quality attributes are actin (thin filament), myosin (thick filament), and their joint structure actomyosin, which are classified as salt-soluble portions (Pearce et al., 2011).

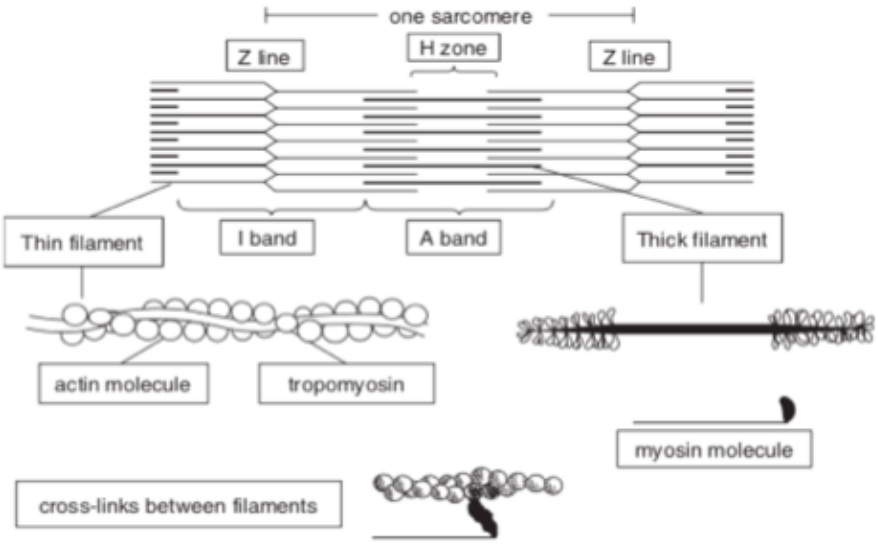

Figure 2.9 Schematic representation of muscle ultrastructure. Source: (Sayas-Barberá et al., 2010).

Approximately 88-95% of water within the muscle is held intracellularly in the space between actin and myosin filaments, and the remainder of water is located between

the myofibrils (Mir et al., 2017). In designating categories for the WHC of meat samples, there are three prevalent terms: water binding potential (WBP), expressible moisture, and free drop. WBP denotes the maximum amount of water that muscle proteins can retain under the conditions prevailing at measurement (Mir et al., 2017). The latter two terms, expressible moisture and free drip, represent the quantity of water that is discharged from the meat by using force and the amount of water lost by the meat without using force other than capillary forces (i.e. gravity), respectively (Mir et al., 2017).

2.7.2 Microbial Status

Throughout the slaughtering process of broiler, cross-contamination can occur when the skin is ruptured (e.g. exsanguination, removal of the head, esophagus and trachea, and excising the vent), equipment comes into contact with the carcasses (plucking, evisceration, and transport to different stages) and carcasses are in contact with water (scalding, washing, and immersion chilling) (Grashorn, 2010). In regards to specific meat surfaces, chicken skin has one of the most optimal surfaces for bacterial attachment. Poultry processors contend with the double-edged sword of bacterial decontamination. The bacteria attached and entrapped firmly are less likely to generate cross-contamination of equipment and other carcasses if they are exposed to chemical disinfectants and washings. In addition, the bacteria are less readily detected by carcass sampling methods (e.g. swabbing and rinsing), hence resulting in a miscalculation of food safety (Oscar, 2008). Bacterial contamination of skin or meat surfaces during processing occurs in two stages: bacteria in the water layer bind then loosely attach to sites on the meat or skin surfaces, via ionic interactions, then the bacteria become firmly attached by producing an extracellular matrix, which subsequently forms a biofilm. The

rate and strength of bacterial attachment depend on several factors such as bacterial strain, physiological state of bacteria, type of meat surface, contact time, and temperature (Oscar, 2008).

Theoretically, salt can decrease bacterial attachment by disrupting the initial stage of attachment; therefore, leading to enhanced removal of microbial cross-contamination during rinsing. As a result, saline rinses can reduce firm attachment of *Salmonella* to chicken skin (Oscar, 2008). Aside from saline concentrations, carcass chilling is one of most critical stages for reducing microbial populations on the product. Despite a site of cross-contamination, the stage of carcass chilling is the place where antimicrobial interventions are added to decrease microbial loads (Grashorn, 2010). Immersion chilling in water or ice slurry mixtures produces a larger reduction in microorganism populations than air or spray chilling methods, as long as the slush ice is replaced periodically (James et al., 2006; Grashorn, 2010). In the case of efficient immersion chilling, it is advised that the total count of microbes in the chilling tank should be less than 5000 colony forming unit (CFC)/ml at 22°C (Grashorn, 2010).

Bacterial attachment is a complex process that cannot only be explained by their surface structures- fimbriae, flagella, and outer membrane/surface polysaccharides (James et al., 2006). Regardless of the possible mechanisms, bacterial attachment to surfaces most likely occurs in two steps: 1) an initial, rapid, reversible, and physical phase followed by (2) an irreversible, molecular, and cellular phase. During the reversible phase, bacterial cells are adjacent to but not in actual contact with the surface. This initial contact between the bacterial cells and the surface is balanced between Lifshitz–van der Waals attraction and electrostatic interactions (James et al., 2006). Due to the presence of

Brownian motion, the bacterial cells can be removed effortlessly with fluid shear forces (i.e. rinsing).

In regards to the electrostatic interactions, the bacterial cells and the muscle surface exhibit a negative charge. The repulsive electrostatic forces likely prevent bacterial cells from creating a firmer attachment to the surface. Yet, bacterial surface structures can function as a bridge between bacterial cells and the surface to overcome this energy barrier; thus, enable an irreversible attachment to the surface (James et al., 2006). At the irreversible phase, there are numerous short range forces such as hydrogen, ionic, covalent bonding and hydrophobic interactions. Accordingly, removing bacterial cells in this phase demands stronger physical forces: scrubbing, scrapping, or chemical treatment with enzymes, sanitizers, detergents, and/or surfactants (James et al., 2006). Once the irreversible surface attachment is assembled, the attached bacterial cells begin to proliferate and generate exopolymeric substances which eventually leads to the formation of a bacterial biofilm.

2.7.3 Temperature Reduction

Microorganisms are inherently adapted for optimum functioning in their normal physiological environments, and any radical change in normal environmental conditions inflicts a stress on an organism (Beales, 2003). One of the most prevalent obstacles that bacteria must overcome is temperature. For bacteria to inhabit and grow in other organisms, it is imperative to have mechanisms that can combat temperature fluctuations in the terrestrial environment (Lim and Gross, 2011). In the case of Gram-negative bacteria, they are considered more sensitive to cold shock, chilling, and freezing than

Gram-positive bacteria (Beales, 2003). Mesophilic bacteria (e.g. *Listeria monocytogenes* and *E. coli*) replicate at temperatures between 20-45°C, with an ideal growth rate at 30-40°C (Simpson and Sofos, 2009).

The growth rate of an organism can be determined as the sum of its chemical reactions; the Arrhenius equation anticipates that there is a linear relationship between the logarithm of the velocity of a chemical reaction and the reciprocal of the absolute temperature (K°C) (Lim and Gross, 2011). In the case of *E. coli*, the normal temperature range encompasses 25°C to 37°C as shown in Figure 2.10. When cells are shifted within the normal growth range, there is little or no lag in adaptation to the new growth rate, and neither a heat shock response (HSR) nor a cold shock response (CSR) is elicited. (Lim and Gross, 2011).

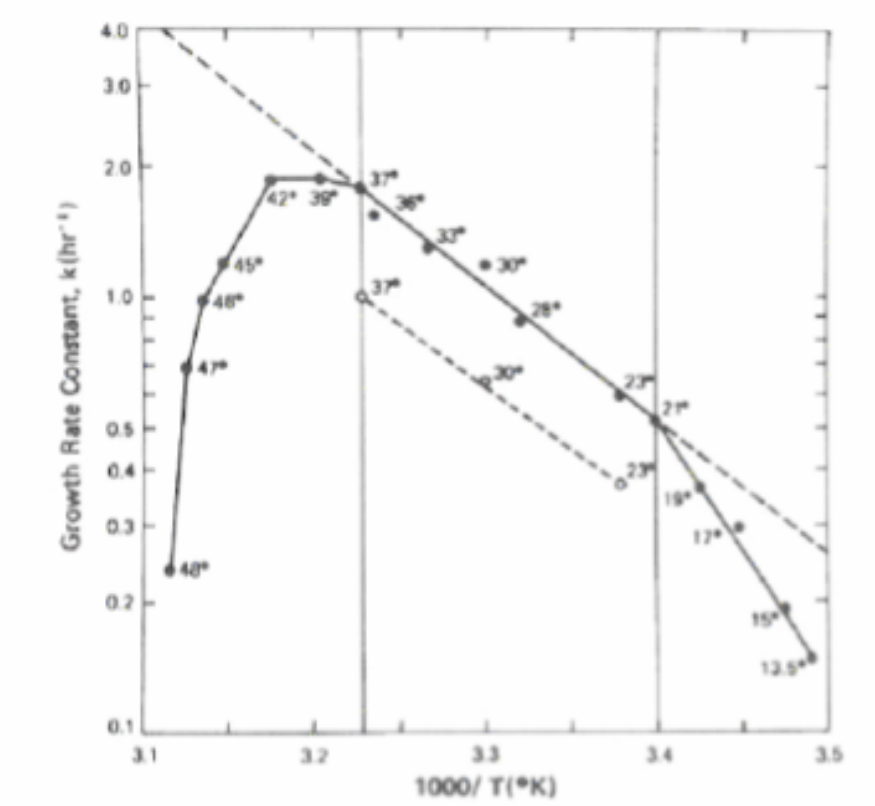

Figure 2.10 Growth rate of *E. coli* as a function of temperature, with specific growth rate (k, hour^{-1}), log scale, is plotted against the inverse of absolute temperature (K). Source: (Lim & Gross, 2011).

In contrast, at successively higher or lower temperatures, the growth rate of bacteria declines until lethal temperatures (high or low) are reached, depending on particular strain and media conditions. Shift from the normal growth range to temperatures above 37°C elicits a progressively more severe HSR; conversely, shift to temperatures of 15°C or below results in a classic CSR (Lim and Gross, 2011). Upon the shift from a temperature within the normal growth range to a low temperature (10-15°C), cells exhibit a "cold shock response," in which growth virtually ceases for several hours before it commences again at a much-reduced rate. The response has distinct three phases: 1) an initial induction phase (also called cold acclimatization), during which only

a small subset of proteins is synthesized, 2) an adaptation phase, where the rate of synthesis of this set of proteins declines and general protein synthesis begins to increase; and 3) a steady-state phase when the cellular complement of proteins is maintained at the low temperature. It is recognized that the CSR exhibits something similar to thermotolerance. When cells are "pre-adapted" to low temperature, a subsequent shift to low temperature reduces the lag time of growth (Lim and Gross, 2011).

When there is a sudden decrease in temperature, the range of ionization of water drops, thus reducing the concentration of H^+ and OH^- ions. Since these ions participate in many biochemical reactions, the change directly impacts reaction velocity. Additionally, there is a simultaneous decrease in the diffusion of water and an increase in its viscosity (Lim and Gross, 2011). These properties directly impact biochemical reactions as well as the diffusion of substrates into the cell, thus poses a severe limitation for growth. Organisms that permanently grow in cold temperatures often have special cold-adapted forms of enzymes. These enzymes often have increased specific activity as a result of decreased activation energy barriers. As mesophilic organisms generally do not have cold-adapted versions of their enzymes, the colder temperature provides more challenges on growth (Lim and Gross, 2011). While the temperature descends below normal range, the lag phase extends prior to growth extends, the growth rate decreases, and the final cell numbers dwindle (Beales, 2003). Within the extended lag phase, certain physiological changes are altered such as decreased fatty acid saturation, DNA/RNA inhibition, and protein synthesis. Moreover, DNA becomes more negatively supercoiled and membrane lipids undergo phase transitions that reduce their fluidity (Lim and Gross, 2011). Additionally, secondary structures in RNA are stabilized and this is likely to affect

multiple cellular processes including transcription, translation, and mRNA decay. Lastly, assembly of ribosomes proceeds poorly at low temperatures. A wide range of evidence suggests that the overwhelming cellular inducer of the CSR is the sudden limitation in translation initiation induced by shift to low temperature.

2.7.4 Salt Concentration

Water activity (a_w) is one of the most critical intrinsic properties in determining the proliferation of microorganisms in food based on its impact on product stability (Tapia et al., 2007). The minimal water activity value is the designated level below which microorganism or group of microorganisms can no longer multiply, despite if others more resistant and adaptable to lower a_w can grow and spoil. Most microbes, with the exception of moderate to highly halophilic strains, proliferate rapidly at the water activity (a_w) range of 0.85 to 0.95 or even 0.98 (Sofos, 1984; Tapia et al., 2007). At moderately low a_w, the lag phase becomes infinite; thus resulting in little to no growth. Consequently, water activity detected below the optimal range results in an extended lag phase and a reduction in growth rate (Sofos, 1984). In particular, a shortage of available water impedes proper metabolic function and cellular division of spoilage, pathogenic, and fermentative microorganisms (Simpson and Sofos, 2009). In spite of inhibiting one microorganism species, this does not guarantee reductions in other microbial populations. Antimicrobials function differently on gram-negative and gram-positive bacteria due to variability in cell membrane and/or metabolic requirements; specifically, gram-negative bacteria have an outer cell membrane containing lipopolysaccharides and gram-positive bacteria do not (Simpson and Sofos, 2009). By adding at least 13% NaCl in chilling solution, the solutes initiate its primary antimicrobial action or cellular dehydration,

which leads to growth inhibition of most pathogenic microorganisms (Simpson and Sofos, 2009). The secondary inhibition usually proceeds after the extraction of extracellular nutrients plus available water (diminishing a_w) from the aqueous environment. This secondary antimicrobial action of NaCl is often considered indirect since it inhibits microbial growth by reducing the a_w of the meat (Sofos, 1984).

Hajmeer et al. (2006) examined the response of two bacterial pathogens, *E. coli* O157:H7 and *Staphylococcus aureus (S. aureus)*, to NaCl by monitoring changes in cell morphology via transmission electron microscope (TEM). The results indicated that *E. coli* O157:H7 cells exhibited more cellular morphological damage, destruction, or alteration with increasing NaCl concentration than *S. aureus*. Observed abnormalities in cell morphology include cell elongation, partial disintegration of cellular membrane, and loss of cellular integrity caused by salt exposure. In fact, as NaCl concentration was increased from 5% to 10% for both salt types (fine or extra coarse), the extent of cellular damage increased; the higher concentration of NaCl resulted in more irregular cell morphology (i.e. cell elongation and partial disintegration of cell membrane) (Hajmeer et al., 2006). Although *E. coli* cells were negatively impacted by the 5% fine salt environment, they were observed as tolerant in 5% fine NaCl compared to 10% concentration, regardless of NaCl type. It was also noted that *E. coli* cells in 10% extra coarse NaCl were not as critically damaged as those in 10% fine NaCl (Hajmeer et al., 2006).

Theoretically, broiler carcasses chilled in sub-zero saline solutions are expected to reduce bacterial activity and attachment to carcasses because of the sub-zero temperature, reduced skin swell, and roles of salt.

Chapter 3

EXPERIMENT: BROILER CARCASS CHILLING IN CONTROL AND SUB-ZERO BRINE SOLUTIONS

3.1 Introduction

In the United States, WIC is a favored method for chilling broiler carcasses in processing plants. The advantages of immersion chilling are efficient heat transfer, no moisture loss, and bacterial reductions (Smith et al., 2005; Demirok et al., 2013). However, the disadvantages of this chilling are high consumption of potable water and high discharge of wastewater, and addition of antimicrobial agents. Current intervention technologies are including inside and outside carcass washers, adding freshwater inflow throughout chilling, continuous counterflow system, and adding antimicrobials to chiller water (Smith et al., 2005). The carcass washers and water addition to the chiller lead to high consumption of water and increase of processing costs, although the counterflow immersion chillers (with clean water inflow at the end of the tank) were superior at decreasing bacterial numbers and cross-contamination. Under the circumstance of water limit, the poultry industry is diligently searching for advanced technologies to enhance processing efficiency and optimize water usage without jeopardizing product safety (Huezo et al., 2007).

In 2001, the USDA published a regulation on moisture retention for post-eviscerated poultry that requires processing facilities to document the amount of water retained in chilled poultry carcasses and carcass parts, and disclose the amount of water on the product label (Huezo et al., 2007). Previous research on water absorption after

WIC has shown that the majority of the water is held between the broiler skin and the muscle, which drips from the carcass during cut-up and deboning process (Jeong et al., 2011; Metheny et al., 2019). Young and Smith (2004) evaluated the moisture retention of immersion-chilled carcasses and found that the carcasses lost 5.7% moisture during the cut-up plus an additional 2.1% during storage. It has been known that immersion chilling is a water intensive process, often needing approximately 2.6 L/bird to fill the chill tank at shift startup and supplemental overflow of 1.9 L/bird (Huezo et al., 2007). Based on previous surveys, the average water usage for poultry processing in the United States is roughly 26.0 L/bird (Kiepper, 2003; Northcutt and Jones, 2004).

Meat tenderness is another complicated issue in addition to processing efficiency and microbial safety. Although mechanical measurement cannot account for different textural characteristics such as chewy, hard, tough, stringy, soft, or mushy, the measurement indicates substantial or lack of tenderness (Kerth, 2013). One accepted method for tenderness measurement is shear force, which is the amount of physical force needed to cut a piece of meat. It is postulated that the meat in shorter sarcomere lengths requires more shear force to cut. The muscles of shorter sarcomeres contain more overlapping of thin and thick filaments and more actomyosin cross-bridges, thus creating a denser sarcomere as compared to fully-extended muscles (Kerth, 2013). Although shear force values are valid measures of protein tenderness, these measurements do not explain differences in textural properties in connective tissue quantity.

Sub-zero saline chilling has the potential to meet the demands of the poultry industry such as improving chilling efficiency, meat tenderness, and product safety. In our previous study, broiler carcasses in high saline solutions (4% NaCl/-2.41°C and 8%

NaCl/-5.08ºC) were chilled significantly faster and resulted in more tenderized meat than the carcasses in conventional water immersion chilling (0% NaCl/0.5 ºC) (Metheny et al., 2019). In the follow-up study, the broiler carcasses chilled in low saline solutions (1% NaCl/-0.6 °C, 2% NaCl/-1.2 °C, and 3% NaCl/-1.8 °C) demonstrated a step-wise increase in tenderness as the salt content was raised from 0 to 3% and the chilling temperature was decreased from 0.5 to -1.8°C (Metheny et al., 2019). In the study, however, the effects of two sub-zero saline solutions (3% NaCl/-1.8°C and 4% NaCl/-2.41°C) have not been compared directly.

Therefore, the purpose of this experiment was to study the effects of two sub-zero saline solutions (3% NaCl/-1.8°C and 4% NaCl/-2.41°C) and one water control (0% NaCl/0.5°C) on broiler carcasses for chilling efficiency, meat tenderness, and microbial safety. It is hypothesized that chilling broiler carcasses in sub-zero saline solutions will increase chilling efficiency, meat tenderness, and microbial safety compared to the those chilled in the control solution. With a higher concentration of salt available to bind with water molecules in the muscle fibers, it can reduce the tension of the actin-myosin interaction by spreading apart the myofibrils; therefore, yielding more tender meat that inhabits pre-rigor qualities.

3.2 Materials and Methods

All procedures were approved by the Institutional Animal Care and Use Committee of California Polytechnic State University (Protocol # 1908).

3.2.1 Brine Chilling Solution and Brine Ice Preparation

Both brine solution and brine ice were prepared using the method of Metheny et al. (2019). Briefly, salt (NaCl) was added and completely dissolved in 20-gallon containers to have the target NaCl concentrations (0, 3 or 4% NaCl) by weight (w/w). After dissolving the NaCl, one-quart Ziploc bags were used to store the saline solutions. The 20 gallon containers and Ziploc bags were then kept overnight in a freezer room at -23°C (Figure 3.1) while the control solution (0% NaCl/0.5°C) was kept in a cooling room at 0.5°C.

Figure 3.1 Saline brine preparation (left: 3% NaCl; right: 4% NaCl)

3.2.2 Broiler Carcass Processing and Chilling

A total of 48 broiler carcasses (Ross 708, roughly 45 days old) were subjected to 12 h feed withdrawal, cooped, and transported from the Poultry Unit to the Meat Processing Center at California Polytechnic State University (Cal Poly). Three replications were conducted with the broilers, thus 16 birds per replication.

Each broiler was placed on the shackle line, electrically stunned for 5 s, and then bled for 90 s after manually severing the carotid artery and jugular vein on one side of the neck. After bleeding, the broilers were scalded at 56.7 ^{o}C for 2 min, defeathered in a rotary drum picker for 1 min, and eviscerated. After rinsing, the broiler carcasses (2.0 kg average weight) were hung on a shackle line for 3 min, tagged on wing, weighed, and randomly assigned to one of the three chilling solutions of 0% NaCl/0.5 ºC, 3% NaCl/-1.8°C and 4% NaCl/-2.41°C.

The broiler carcasses were chilled with mechanical agitation in the chilling solution (Figure 3.2A). During chilling, one medium carcass per treatment was used for monitoring the internal breast temperature every 5 min until the carcass temperature reached approximately 4°C using a digital thermometer logger (ThermaData Thermocouple Logger KTC, ThermoWorks, American Fork, UT) (Figure 3.2B). Throughout chilling, supplemental control ice and brine ice were added to maintain the target solution temperatures. Once chilling was completed, carcasses were hung on a shackle for 3 min, weighed, and stored in the poultry cooler (1.1 °C) until 3 h post mortem.

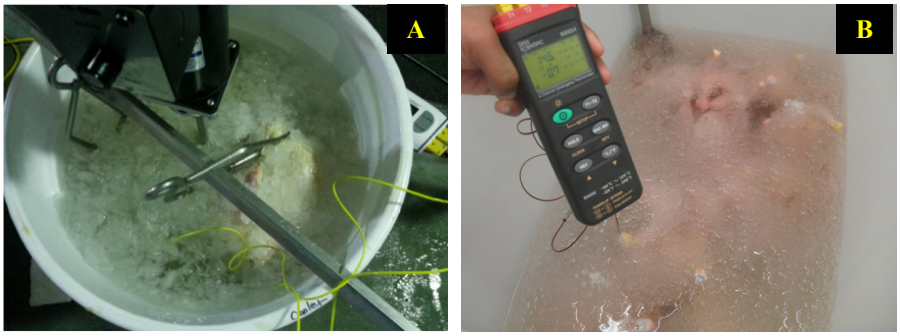

Figure 3.2 Mechanical agitation (A) and temperature monitoring (B) during chilling of broiler carcasses.

3.2.3 Breast Fillet Cooking Yield and Shear Force

The breast fillets were excised from each carcass at 3 h post mortem. The right fillets from each carcass were placed into individual Ziploc bags and held in a cooler room (2.2°C) for 24 h. The left fillets were divided into two cranial and one caudal pieces, inserted into Ziploc bags individually, and immediately quick frozen (IQF) via liquid nitrogen. The following day, the right breast fillets were weighed for a pre-cooked weight, wrapped in aluminum foil and arranged on stainless trays. The breast fillets were covered with aluminum foil to cook to an internal temperature of 76.7°C in a convection oven (36S-Y1A Wolf Challenger XL Range, ITW Food Equipment Group LLC, Gleenview, IL) (2019). After cooking, the fillets were weighed for post-cook weight. Cooking yield was calculated using the formulation: (post-cook weight)/(pre-cook weight) × 100.

Shear force was determined by the razor-blade method of Cavitt et al. (2004), using a texture analyzer (TAHDi, Texture Technologies Corp., Scarsdale, NY) calibrated with a 25-kg load cell. The razor blade (height, 24 mm; width, 8 mm) was set at 10 mm/s, and the test was triggered by a 10-g contact force. The shear force value (N) was determined as the maximum force recorded during the actual shear. Two shear force measurements were obtained for each breast fillet.

3.2.4 pH, R-value, and Sarcomere Length

For pH measurement, the frozen cranial piece (2.5 g) of the left breast fillet was pulverized and homogenized with 25 mL of 5 M iodoacetate solution with 150 M

potassium chloride for 30 s following the method of Sams and Janky (1986). The pH of the homogenate was measured by using a pH electrode, attached to the pH meter.

R-value, the ratio of inosine to adenosine, was evaluated as an indicator of adenosine triphosphate (ATP) levels in the muscle by utilizing the method of Thompson (1987). The remaining cranial piece (3 g) of the left breast fillet was pulverized and homogenized with 20 mL of 1M perchloric acid solution for 1 min, then filtered through Fisher P8 filter paper. R-value was measured using the absorbance via spectrophotometer at 250 nm (IMP) and 260 nm (ATP) after filtrating (0.2 mL) the sample mixture with 8.0 mL of 0.1M phosphate buffer.

Sarcomere length was assessed as the status of muscle contraction by using the laser diffraction method (Cross et al., 1981). The caudal piece (10-15 g) of the left breast fillet was homogenized in a 50 mL solution comprised of 0.25 M sucrose, 2 M potassium chloride, and 5 M sodium iodoacetate. When homogenization was complete, a drop of homogenate was added onto a slide, with a cover slip on the laser platform. The slide was adjusted until a diffraction pattern appeared, at which the measurement was taken. The diffraction measurement was converted into sarcomere length based on the equation shown below in Figure 3.3 (Metheny et al., 2019).

$$Sarcomere\ Length\ =\ \frac{0.6328 * \mathrm{D} * \left[(\frac{T}{D})^2\ +\ 1\right]^{\frac{1}{2}}}{T}$$

0.6328 = Wavelength of the Helium-Neon laser light

D = Distance in mm from specimen to the diffraction screen

T = Distance in mm from the origin to the first order diffraction band

Figure 3.3 Sarcomere length equation. Source: (Metheny et al., 2019)

3.2.5 Microbial Analysis

For microbial test, the amount (10 g) of breast skin was aseptically excised using a sterile scalpel and a forceps. The excised skin was transferred to a clean WhirlPakR® bag, filled with 90 mL sterile PBS, and stomached for 1 min. Following the stomaching, serial 10-fold dilutions were made, and surface plating (1 mL) was made in duplicate on Petrifilm™ (3M Microbiology Products, St. Paul, MN) for mesophilic aerobic bacteria (MAB). For *E. coli* and coliforms, the 10-fold diluted samples were similarly plated (1 mL) on Petrifilm™ *E. coli*/coliform count plates. All samples were incubated at 37°C for 24 h prior to enumeration. Results were expressed as log colony forming units (CFU) per gram of skin.

3.2.6 Statistical Analysis

Data in this experiment were statistically analyzed by one-way ANOVA, using PASW 18 statistic program and a completely randomized design. A post-hoc analysis

was performed using Duncan's multiple range test to evaluate differences among treatments ($P < 0.05$; SPSS, 2011).

3.3 Results and Discussion

3.3.1 Broiler Carcass Chilling

Prior to chilling, the internal temperature of the eviscerated carcasses was approximately 40ºC, which was continuously reduced to 4.3-4.5ºC, with average chilling times of 90, 80, and 55 min for the water control (0% NaCl/0.5 ºC) and two brine solutions (3% NaCl/-1.8 ºC and 4% NaCl/-2.4 ºC), respectively (Figure 3.4). Compared to the water control, 4% and 3% NaCl solutions reduced the broiler chilling time by 39 % and 11%, respectively. Previously, Metheny et al. (2019) reported a chilling time reduction of 22% when the 3% NaCl/-1.8 ºC solution was used over the water control. This 11% reduction in chilling efficiency between the control and 3% NaCl solutions could be attributed to carcass size; the birds used in Metheny et al. (2019) were about 1.5 kg, whereas the birds used in this study weighed between 2.9-3.2 kg.

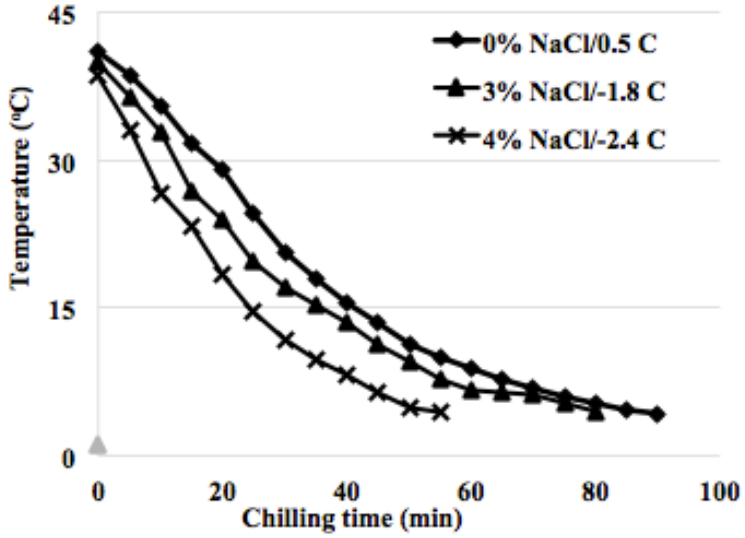

Figure 3.4 Temperature change profiles of broiler fillets during chilling[a] in water and brine solutions.

[a]0% NaCl/0.5°C; Carcass chilling in ice slurry (0% NaCl) at 0.5°C.
3% NaCl/-1.8°C; Carcass chilling in 3% NaCl at -1.8°C.
4% NaCl/-2.4°C; Carcass chilling in 4% NaCl at -2.4°C.

Previously, Jeong et al. (2011b) reported that the average chilling time of WIC was 55 min. Likewise, Huezo et al. (2007) observed that an internal carcass temperature was reduced to 4.4 °C in 50 min in immersion chilling. In comparing the control and saline chilling solutions, the brine solutions showed higher chilling rates because of the sub-zero temperature. In general, saline chilling media have more efficient heat and mass transfer properties than water control media due to the freezing point depressant (i.e. sodium chloride) (Meewisse and Ferreira, 2001). Sodium chloride has a low molecular weight that is anticipated to yield more efficient ice slurries because it lowers the freezing point; thus, the ice slurry transfers heat by melting the ice, without raising the solution temperature.

3.3.2 Shear Force

Tenderness is one of the crucial determining factors in consumer acceptance and discernment of palatability (Cavitt et al., 2005). Shear force was selected as the measurement to evaluate tenderness of broiler breast fillets with the higher shear value for the tougher fillet (Demirok et al., 2013). Breast fillets obtained from carcasses chilled in 4% NaCl/-2.4 ºC solution showed significantly reduced shear force (8 N) than that (12.64 N) of breast fillets from broilers in the control solution, with the intermediate (10.15 N) observed for the fillets in 3% NaCl/-1.8 ºC solution (Figure 3.5). Shear force values lower than 10 N are considered tender. The lower shear force value is probably due to less heat shortening or muscle contraction.

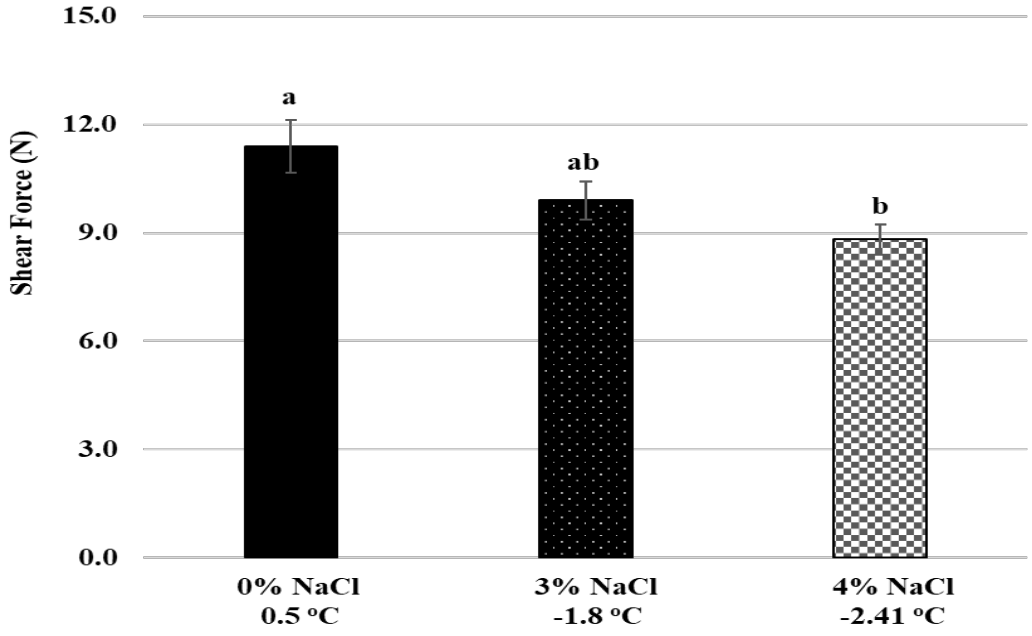

Figure 3.5. Effects of broiler chilling[a] temperature and salt content on shear force (SF) of broiler breast fillets

Chilling conditions as in Figure 1.
[1]Number of observation, n = 12

These results are similar to the trend of previous research (Metheny et al., 2019), which suggests that breast fillets become more tenderized as the salt content increased (0% to 8% NaCl) and the solution temperatures reduced (0.5 ^{o}C to -5.1 ^{o}C) during chilling. Sams et al. (1986) evaluated the shear forces of cooked breast fillets of broiler carcasses that were chilled in water, NaCl, potassium chloride (KCl), and Neobakasal® (a commercial salt substitute). Results showed that the shear force values in the breast fillets in NaCl and KCl were significantly lowered than those of Neobakasal® ($P < 0.05$). Although the shear force value in the Neobakasal®-chilled carcasses was 2.94 N higher than the NaCl- and KCl-chilled carcasses, it was still 7.85 N lower than that of water-chilled controls. The researchers indicated that the tenderization in brine chilling is caused by enhanced WHC, which was produced by the increased chloride ion in the muscle tissue during the chilling (Sams et al., 1986). This coincides with the high water uptake of the carcasses in the three salt solutions (0.8 to 1 percentage points) as compared to the control carcasses chilled in water. Janky et al. (1978) evaluated the tenderness of broiler carcasses chilled in one of the three treatments: 1) hot-packaging with no chilling, 2) ice slush at 1°C overnight, and 3) 5% NaCl ice slush at -1°C overnight. The results showed that the shear force was significantly lower in the breast meats of carcasses in brine chilling than those of water-chilling or no chilling.

In the study of Zhuang et al. (2008), broiler carcasses were randomly assigned to one of three chilling methods (AC, WIC, or no chill/hot-boned). The results showed no difference in shear force between AC and WIC samples (45.7 vs. 44.7 N and 21.6 vs. 20.7 N) for *P. major* and *P. minor* muscles at 4 h post-mortem, respectively. However, there was a significant difference between hot-boned muscles and chilled muscles for

50% in *P. major* and 30% in *P. minor* fillets. These results suggest that air-chilling did not improve the shear force of the broiler breast muscles in comparison to immersion-chilling (Zhuang et al., 2008).

Cavitt et al. (2004) examined the mean razor blade shear force measurements of broiler breast fillets, derived from WIC carcasses, that were deboned from 0.25 to 24 h postmortem. As the time intervals progressed from 1.25 h (13.81 N) to 3.5 h (11.82 N) to 6 h (10.47 N), the shear force values decreased and sarcomere lengths became larger/less contracted. Generally speaking, the breast fillet samples deboned from 3.5 h to 24.0 h postmortem demonstated little variation in tenderness; in particular, the 6.0 h and 24.0 h treatments did not signficantly differ for any sensory quality. In addition, a trained sensory panel evaluated the same meat samples for attributes such as initial hardness (the force required to compress the samples) and cohesiveness (the amount the sample deforms rather than splits apart, cracks, or breaks). The mean descriptive sensory texture scores of broiler breast fillets deboned at different postmortem times exhibited a similar pattern to that of the mean shear force values. Broiler breast samples deboned at 0.25 h were not significanlty different for initial hardness and cohesiveness than samples deboned at 1.25 h until 3.0 h postmortem. However, the samples showed lower initial hardness and cohesiveness values as deboning time progressed to 24.0 h postmortem, likely due to rigor mortis development and ATP breakdown. Furthermore, the sensory panelists were able to discern differences between breast fillet samples deboned early postmortem (<3.0 h) and late postmortem (>3.0 h).

3.3.3 pH, R-value, and Sarcomere Length

Broiler breast muscle pH and R-value were measured to evaluate the status of post-mortem metabolism in the muscle. The speed of reduction and final pH of the broiler breast muscle are the key indicators of meat quality as they are linked to the rate of glycogen depletion as well as lactic acid accumulation post-slaughter (Mothershaw et al., 2009). A higher pH value indicates less rigor mortis development, but the remaining ATP in the muscle will be depleted as rigor mortis progresses and post-mortem time increases (Zocchi and Sams, 1999). R-value serves as an indirect measure of ATP depletion, which is quantified as the ratio of inosine (degradation product of ATP) to adenosine nucleotides (an element of ATP) (Cavitt et al., 2005).

After chilling, breast fillets were measured for pH, R-value, and sarcomere length. The breast fillets had pH and R-values from 5.85 to 5.94 and 1.0 to 1.15, with no significant differences among the chilling solutions ($P < 0.05$) (Table 1). The sarcomere length (1.97 - 2.02 μm) of broiler carcasses in saline solution was significantly longer than that (1.57 μm) of the control carcasses in 0% NaCl/0.5 ºC, indicating that the fillets in saline chilling were less contracted and likely more tenderized ($P < 0.05$) (Table 1).

Table 1. Evaluation of pH, R-value, and sarcomere length after chilling carcasses in three different chilling solutions.

Chilling	0%NaCl/ 0.5°C	3%NaCl/ -1.8°C	4% NaCl/ -2.4°C
pH	5.94 ± 0.06[a]	5.85 ± 0.14[a]	5.90 ± 0.13[a]
R-value	1.00 ± 0.11[a]	1.15 ± 0.17[a]	1.14 ± 0.20[a]
Sarcomere length (μm)	1.57 ± 0.15[b]	1.9 7 ± 0.09[a]	2.02 ± 0.07[a]

[a-b]Means within a row with no common superscripts are different ($P < 0.05$).
[1]Number of observation, n = 12

The sarcomere is the repeating structural unit of the myofibril and the basic unit involved in the muscular contraction-relaxation cycle (Cavitt and Sams, 2003). Sarcomere length, is closely linked with ATP consumption, and was selected to measure the contractile state of the breast fillet. A severe pH decline, caused by the denaturation of sarcoplasmic and myofibrillar proteins, can trigger more permanent actomyosin cross bridges in an overlapped conformation and produce a tougher meat structure (Yu et al., 2005). In this study, the sarcomere lengths of breast fillets were significantly longer in sub-zero saline solutions (1.9 and 2.02 μm) than those (1.57 μm) in water solution ($P <$ 0.05), supporting that the meat in sub-zero saline solutions were less contracted potentially due to less rigor and/or cold shortening. (Table 1).

3.3.4 Microbial Analysis

Before chilling, the excised skin of broiler carcasses showed the populations of mesophilic aerobic bacteria (MAB), *Escherichia coli* (*E. coli*), and total coliforms at 3.81, 0.78, and 1.86 log cfu/g, respectively (Table 2). After chilling, the *E. coli* and total

coliforms were significantly reduced on the carcasses chilled in 3% NaCl/-1.8 °C or 4% NaCl/-2.41°C than the water control ($P < 0.05$) (Table 2). No statistically significant difference was detected for MAB, regardless of chilling solution (Table 2). Given these results, the combination of sub-zero temperatures and 4% NaCl solution might generate an unfavorable condition, especially to Gram-negative bacteria.

Table 2. Mean population[1] (log cfu/g) of mesophilic aerobic bacteria (MAB), *Escherichia. coli*, (*E. coli*) and total coliforms on broiler skin after chilling.

	Before chilling	After chilling		
	None	0%NaCl/ 0.5 °C	3% NaCl/ - 1.8 °C	4% NaCl/ -2.4 °C
MAB	3.81 ± 0.09[a]	3.62 ± 0.13[ab]	3.49 ± 0.14[ab]	3.34 ± 0.15[b]
E. coli	0.78 ± 0.20[a]	0.60 ± 0.13[a]	0.02 ± 0.02[c]	0.23 ± 0.09[bc]
Coliforms	1.86 ± 0.12[a]	1.34 ± 0.15[b]	0.37 ± 0.12[c]	0.63 ± 0.13[c]

In a commercial processing plant, five carcasses were randomly selected prior to entering the chilling system while additional five carcasses were selected at the exit of chill tank after first (8 h) and second (16 h) shifts (Souza et al., 2012). Each sample (25 g) of the muscle and skin was obtained for microbial testing from the pericloacal area, thighs, wings, and neck of the selected carcasses. There were significant drops in the bacterial counts for aerobic mesophiles, coliforms, and *E. coli* after transitioning through the chilling system after the first and second shifts. These results were expected since the chilling tanks were not emptied, cleaned, and disinfected prior to the shifts.

It is hypothesized that the reductions observed in the microbial populations were due to the counter-flow movement of carcasses against the flow of water in the chill tanks. The current of carcasses flowing opposite to the agitated water causes mechanical removal of bacteria on the birds while the renewal of chilling solution in the tanks promotes the removal of microorganisms from the immersion system (Souza et al.,

2012). Demirok et al. (2013) examined microbial populations on whole carcasses after immersion chilling (IC), air chilling (AC), and combination in-line air chilling (CIAC; similar to evaporative air chilling). The aerobic plate count for AC and CIAC intact carcasses were not significantly different ($P > 0.05$) at 1, 5, and 10 days post-mortem; however the IC carcasses showed significantly lower for all three time periods. Upon examining boneless skinless breast fillets subjected to the different chilling methods, the CIAC samples at 0 day had the highest aerobic plate count (3.88 log cfu/mL) than the AC samples (3.57 log cfu/mL), with the significantly lowest aerobic plate count (2.97 log cfu/mL) in IC samples ($P > 0.05$). At 10 days, the breast samples from all treatments showed greater than 6 log cfu/ml, meaning no significant difference in aerobic plate counts, and the samples were deemed spoiled ($P > 0.05$). There was no significant difference between IC and AC samples at 5 days, but there was a difference between the two treatments and CIAC samples (Demirok et al., 2013).

In contrast to those results, Carroll and Alvarado (2008) observed significantly lower aerobic plate counts and lower coliform counts with AC samples compared to IC samples, proposing that AC systems reduce bird to bird cross contamination by chilling an individual carcass per shackling line. In the study of brine-chilling, water-chilling, and non-chilling, Janky et al. (1978) reported that one log reduction was found for aerobic and coliform bacteria on the brine-chilled birds compared to no-chilling or water-chilled birds.

In spite of the improved chilling efficiency, meat tenderization, and microbial safety, saltwater pickup after sub-zero saline chilling is a challenging outcome. It will be ideal to minimize the salt pick up in the carcasses and salt discharge to the waste stream.

Poultry processing facilities are mandated to record the amount of water retained in chilled carcasses and subsequent cut ups, which should be notified on the product label (Northcutt et al., 2006). It is theorized that carcasses immersed in the chilling tank can absorb water until the intercellular spaces are partially filled. It is after 10-15 min of chilling that the second stage of water absorption occurs, where the rate of liquid uptake declines substantially due to internal pseudo-diffusion of water in the muscle (Carciofi and Laurindo, 2007). According to James et al. (2006), the percentage of ice incorporated into ice slurry systems has been revealed to influence moisture absorption by the carcass and chilling time. Besides the ratio of ice to water, it is imperative for the poultry processing industry to ascertain the major variables (e.g. maximum chill tank entrance and exit water temperature, number of tanks, minimum stream flow, and more) that affect water absorption by carcasses during chilling (Martins et al., 2011).

Chapter 4

CONCLUSIONS

In summary, the chilling time of broiler carcass significantly reduced from 90 min in water control (0% NaCl/0.5 ºC) to 55 min in 4% NaCl/-2.4 ºC, demonstrating the improvement of chilling efficiency by 39%. Although there was no significant difference in fillet pH between sub-zero saline chilling and water control chilling, there was significantly longer sarcomere lengths for the sub-zero saline solutions (3% NaCl/-1.8 °C and 4% NaCl/-2.41°C) than the control ($P < 0.05$). Breast fillet tenderness was significantly improved as the salt concentration increased from 0 to 4% and chilling temperature decreased from 0.5 to -2.4ºC ($P < 0.05$), indicating that the meat was tenderized with longer sarcomere lengths, presumably due to less hot shortening and cold shortening during the rapid chilling. There was significantly lower *E. coli* and total coliforms populations for both sub-zero saline solutions compared to the control ($P < 0.05$), probably due to the sub-zero temperatures and unfavorable saline environment (Table 2). As a result, the chilling of broiler carcasses in the 4% NaCl/-2.4 °C solution seems to improve chilling efficiency, meat tenderness, and bacterial reduction compared to the control (0% NaCl/0.5ºC) chilling solution.

Chapter 5

AREAS FOR FUTURE STUDY

Sub-zero saline chilling of broiler carcasses demonstrated to improve broiler chilling efficiency, meat tenderness, and bacterial reduction, especially in 4% NaCl/-2.41°C solution. These results could be helpful for poultry processors, environmental engineers, agricultural marketers, and government regulatory members who look for a new technology to improve product quality and safety. Additional research is required for the improvement of water saving, processing cost saving, and salt discharge reduction. One potential experiment is a sensory analysis of the breast fillets, conducted with trained sensory panelists and scored on a 9-point hedonic scale. In addition, monitoring the salt absorption by broiler carcasses chilled in sub-zero saline solutions. Another potential study is a microbial analysis for *Salmonella* and *Campylobacter* populations detected on broiler carcasses chilled in sub-zero saline solutions. This possible study is particularly important because these microorganisms are commonly found in poultry carcasses and processed meats.

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