

University of Alberta

Protein Functionality in Turkey Meat

by

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ABSTRACT

Turkey with pale, soft, exudative (PSE)-like condition is one of the growing concerns in the poultry industry as it affects meat quality due to low ultimate pH at 24 h *post mortem* (pH₂₄). Hence, there is a need for better utilization of PSE-like meat for the preparation of further processed products. In the first two studies, the biochemical, functional, rheological, and textural properties of proteins in turkey breast meat with different pH₂₄ in fresh and frozen conditions were investigated. These studies revealed that low and normal pH meat had similar properties indicating similar extent of protein denaturation, except for lower water holding capacity (WHC) in low pH meat. High pH meat had similar or better functional properties than normal pH meat. In the third study, improvements in WHC, protein solubility, and gel forming ability of low pH meat was achieved by the application of high pressure processing (HPP).

Keywords: PSE, DFD, Turkey, Ultimate pH, Freezing, Biochemical and functional properties, Textural and rheological properties, High pressure processing.

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LIST OF ABBREVIATIONS

- ANOVA: Analysis of variance
- ANS: 1-anilino-8-naphthalene-sulfonate
- ATP: Adenosine triphosphate
- BSA: Bovine serum albumin
- Ca²⁺-ATPase: Calcium activated adenosine triphosphatase
- DFD: Dark, firm, dry
- DHPR: Dihydropyridine receptor
- DNPH: 2,4-dinitrophenylhydrazine
- DVB: Dynamic viscoelastic behavior
- EAI: Emulsion activity index
- EDTA: Ethylenediaminetetraacetic acid
- EM: Expressible moisture
- ESI: Emulsion stability index
- FE: Foam expansion
- FS: Foam stability
- HSD: Honestly significant difference
- HPP: High pressure processing
- L*: Lightness
- LVR: Linear viscoelastic region
- MHC: Myosin heavy chain
- MLC: Myosin light chain

MPH: Myofibrillar protein surface hydrophobicity

NAM: Natural actomyosin

NMR: Nuclear magnetic resonance

PC: Principal components

PCA: Principal component analysis

pH₁₅: pH at 15 min *post mortem*

pH₂₄: Ultimate pH at 24 h *post mortem*

Pi: Inorganic phosphorous

pI: Isoelectric point

PLA₂: Phospholipase A₂

PSE: Pale, soft, exudative

PSS: Porcine stress syndrome

RFI: Relative fluorescence intensity

R-SH: Reactive sulfhydryl groups

RYR: Ryanodine receptor

SAS: Statistical analysis system

SDS-PAGE: Sodium dodecyl sulfate - polyacrylamide gel electrophoresis

SPH: Sarcoplasmic protein surface hydrophobicity

SPS: Sarcoplasmic protein solubility

TCA: Trichloroacetic acid

TnI: Troponin I

TPA: Texture profile analysis

TPS: Total protein solubility

T-SH: Total sulfhydryl groups

WHC: Water holding capacity

CHAPTER 1. INTRODUCTION

Global turkey production has been growing steadily worldwide over the past few decades. There is a projected increase of 2.3% in the world poultry meat production by 2015 (Poultry Marketplace, 2010). This increase is primarily due to a shift in consumers' taste preferences towards leaner meats like chicken and turkey (Poultry Marketplace, 2009). The consumption of white meat is often perceived as a healthier choice when compared to red meat. In Canada, turkey consumption (on a per capita basis) has increased by 31% during 1986 - 2006, which was in part due to growth in population as well as increasing consumer demand for broader diversity of products in the commercial food service industry. As a result, turkey sales are shifting from whole turkeys to further processed turkey products (Poultry Marketplace, 2009).

Protein functionality has an important role in the preparation of further processed products. Functional properties of proteins are the physicochemical properties which determine their behavior during processing, storage, and consumption (Smith, 2001). Some of the important factors which affect functional properties include freezing conditions and pH of meat, which in turn may have an influence on overall product attributes (i.e. textural properties). There are major challenges in the turkey industry due to an increasing occurrence of a pale, soft, exudative (PSE)-like meat condition in turkeys, similar to the one observed in pigs which affects important meat quality attributes. The PSE problem has been studied in the pork industry for the past three decades, while it has only been a focus for the past decade in the turkey industry (Barbut et al., 2005). PSE meat is

characterized by its pale color, soft texture, and exudative surface, and has low pH (Santos et al., 1994; Cassens, 2000). It has been shown that PSE-like chicken and turkey has reduced functionality, exhibiting decreased WHC and weaker gel forming ability (Barbut, 1997; Zhang and Barbut, 2005). Thus, PSE meat causes undesirable characteristics in processed products. The reduced functionality in PSE meat may affect the suitability for the production of processed products. Consequently, the PSE problem has led to substantial economic loss, costing at least U.S. \$2 to \$4 million per year in a single turkey processing plant, resulting in a total loss of at least U.S. \$200 million per year by the turkey industry (Owens et al., 2009). Factors contributing to the development of PSE-like poultry meat include environmental temperature, pre-slaughter handling practices, chilling regimes, and feed quality (Rathgeber et al., 1999; Petracci et al., 2004; Betti et al., 2009; Owens et al., 2009; Zhang et al., 2011). Conversely, dark, firm, dry (DFD) meat condition is another meat quality condition which results in meat with higher pH than normal meat (Guardia et al., 2005). The major concerns with DFD meat are shorter shelf life and darker appearance, which may affect the color of processed products and consumer acceptability (Allen et al., 1997). In order to reduce economic loss due to reduced functionality in PSE meat, there is a need for industry to implement different approaches for improving the functionality.

1.1 REFERENCES

- Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. *Poult. Sci.* 76:1042-1046.
- Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. *Br. Poult. Sci.* 38:355-358.
- Barbut, S., L. Zhang, and M. Marcone. 2005. Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poult. Sci.* 84:797-802.
- Betti, M., B. L. Schneider, W. V. Wismer, V. L. Carney, M. J. Zuidhof, and R. A. Renema. 2009. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poult. Sci.* 88:1085-1095.
- Cassens, R. G. 2000. Historical perspectives and current aspects of pork meat quality in the USA. *Food Chem.* 69:357-363.
- Guardia, M. D., J. Estany, S. Balasch, M. A. Oliver, M. Gispert, and A. Diestre. 2005. Risk assessment of DFD meat due to pre-slaughter conditions in pigs. *Meat Sci.* 70:709-716.
- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Petracci, M., M. Betti, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. *Poult. Sci.* 83:2086-2092.
- Poultry Marketplace. 2009. Profile of the Canadian turkey industry.

http://www.agr.gc.ca/poultry-volaille/prindt_eng.htm

Poultry Marketplace. 2010. World future outlook of the chicken meat market.

http://www.agr.gc.ca/poultry/prindc2_eng.htm.

Rathgeber, B. M., J. A. Boles, and P. J. Shand. 1999. Rapid postmortem pH decline and delayed chilling reduce quality of turkey breast meat. *Poult. Sci.* 78:477-484.

Santos, C., L. C. Roserio, H. Goncalves, and R. S. Melo. 1994. Incidence of different pork quality categories in a Portuguese slaughterhouse: A survey. *Meat Sci.* 38:279-287.

Smith, D. M. 2001. Functional properties of muscle proteins in processed poultry products. Pages 181-194 in *Poultry Meat Processing*. A. R. Sams, ed. CRC Press, Taylor & Francis, Boca Raton, FL, USA.

Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.

Zhang, W., S. Xiao, E. J. Lee, and D. U. Ahn. 2011. Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *J. Agric. Food Chem.* doi: 10.1021/jf102918z.

CHAPTER 2. LITERATURE REVIEW

2.1 Structure and protein composition of muscle

2.1.1 Muscle structure

The skeletal muscle is a complex structure composed of individual muscle fibers. A schematic diagram of a muscle structure is shown in Figure 2.1. A single skeletal muscle is surrounded by the epimysium, which is a thin layer of connective tissue extending from the tendon (Figure 2.1a). Each muscle is composed of muscle fiber bundles (Figure 2.1b), which is covered by the perimysium, another thin layer of connective tissue (Figure 2.1a). In turn, each muscle fiber bundle is composed of individual muscle fibers (Figure 2.1c), which is covered by another membrane of connective tissue, the endomysium (Figure 2.1a). Each muscle fiber consists of myofibrils (Figure 2.1d), which are made up of myofilaments, actin (thin filament) and myosin (thick filament) (Figure 2.1e) (Cassens, 1994). The overlapping arrangement of myofilaments results in dark (A) and light (I) bands. The A band is the area in which actin and myosin overlap. The area in the A band which contains no thin filaments is the H zone while I band is the area which contains no thick filaments (Feiner, 2006). I bands are bisected which results in dark lines known as Z-lines, while bisected A bands are known as M-lines (Toldrá, 2002). The contractile unit of a muscle fiber is the sarcomere, which is located between two Z lines and is approximately 2.5 μm long. Actin and myosin are connected to the Z line and M line, respectively. Muscle fibers have a striated appearance due to the special arrangement of actin and myosin. Myofilaments are attached to the cell membrane called the

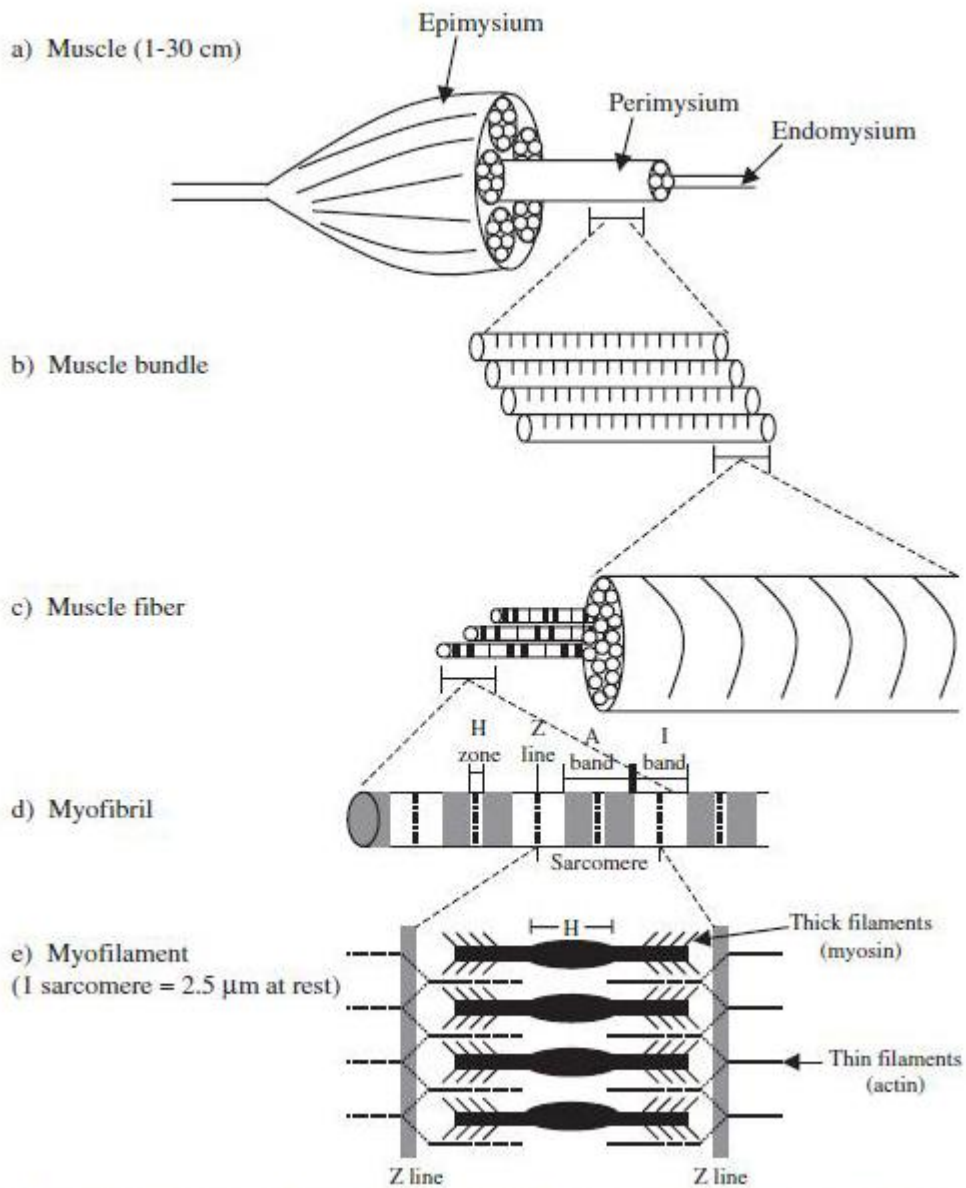


Figure 2.1. Schematic diagram of the muscle structure. (a) Muscle showing different layers of connective tissue, (b) Muscle bundle, (c) Muscle fiber, (d) Myofibril, and (e) Myofilaments. Reprinted from POULTRY PRODUCTS PROCESSING: AN INDUSTRY GUIDE. EBOOK by S. Barbut. Copyright 2001 by Taylor & Francis Group LLC. Reproduced with permission of Taylor & Francis Group LLC in the format of Dissertation via Copyright Clearance Center.

sarcolemma, which has a net-like structure. Muscle fibers are filled with intracellular substance, sarcoplasm (cellular fluid), which is a liquid composed of approximately 80% water as well as proteins, enzymes, lipids, carbohydrates, and inorganic constituents (Aberle et al., 2001).

2.1.2 Muscle protein composition

Lean muscle tissue contains approximately 70 - 75% water, 22% protein, 2 - 4% intramuscular fat, and 2% of other components, such as phosphates and minerals. The 22% protein is generally categorized into three main groups: sarcoplasmic (7%), myofibrillar (13%), and stromal or structural proteins (2%) (Feiner, 2006). This classification is based on their function in a muscle and solubility in aqueous solvents. Sarcoplasmic proteins contribute to approximately 30 - 35% of the total proteins in skeletal muscle and are located in the sarcoplasm. They are water soluble proteins, which include hemoglobin, myoglobin, cytochromes, and glycolytic enzymes (Wang, 2006).

Myofibrillar proteins contribute to approximately 45 - 50% of the total proteins in skeletal muscle and are salt soluble proteins. Myofibrillar proteins can be divided into three main categories: contractile proteins (myosin and actin), regulatory proteins (tropomyosin and troponin), and cytoskeletal proteins (titin, nebulin, C-protein, H-protein, and M-protein). Myosin and actin, the two most abundant contractile proteins, contribute to approximately 55 and 23% of the myofibrillar proteins, respectively, and they play a role in muscle contraction and relaxation. Myosin, the main constituent of the thick filament, is a long rod shaped protein with two globular heads at one end. The two globular heads are

approximately 15 - 18 nm long and 4.5 nm in diameter and are composed of six subunits, which include two heavy chains and four light chains. The rod portion is approximately 135 - 140 nm long and 1.5 nm in diameter and is composed of α -helix (Whitaker and Tannenbaum, 1977). The two globular heads are relatively hydrophobic and are able to bind to actin, while the rod portion is relatively hydrophilic and responsible for the assembly of myosin into thick filaments (Xiong, 1997). Myosin has a molecular weight of approximately 540,000 Da (Barbut, 2002). Actin is the main constituent of the thin filaments. Actin exists in two forms, a globular form (G-actin) and a fibrous form (F-actin). G-actin has a molecular weight of approximately 43,000 Da and is the monomer of F-actin, which has a higher molecular weight (Ramakrishnan et al., 2001). G-actin is required to polymerise to form the fibrous F-actin, which is two chains of actin twisted together forming the double helix. Tropomyosin, a constituent of the thin filaments, surrounds the chains of actin. It is a rod shaped protein and consists of approximately 6% of the myofibrillar proteins. Troponin, another constituent of the thin filaments, is a globular shaped protein. It consists of approximately 6% of the myofibrillar proteins. There are three types of troponin, which include troponin-C (TpC; calcium-binding troponin), troponin-T (TpT; tropomyosin-binding troponin), and troponin-I (TpI; inhibitory troponin), and together they form the troponin-complex. Troponin-C contains calcium binding sites, while troponin-T interacts with tropomyosin and troponin-I blocks the actin binding site for myosin. The binding of calcium (Ca^{2+}) to troponin-C disrupts the binding of troponin-T to tropomyosin, which causes a conformational change and myosin

binding sites on actin becomes exposed (Urich, 1994). This leads to the formation of cross-bridges between actin and myosin, resulting in the actomyosin complex (Whitaker and Tannenbaum, 1977).

Stromal proteins contribute to approximately 10 - 15% of the total proteins in skeletal muscle and are water- and salt-insoluble proteins. Stromal proteins mainly include connective tissue proteins, which provide strength and protection for muscle tissue. There are two types of connective tissue proteins: proper and supportive. The three connective tissue layers that surrounds the muscle (epimysium), muscle bundle (perimysium), and muscle fiber (endomysium) are known as connective tissue proper (Alvarado and Owens, 2006). Supportive connective tissue which includes bones and cartilages provides structural support. The two major stromal proteins are collagen and elastin (Barbut, 2002). Stromal proteins can also be found in the extracellular matrix, which is fibrous in structure. The extracellular matrix is part of three connective tissue layers and is composed of proteins including glycoproteins, proteoglycans, and collagen. Proteoglycans are a multifunctional component of the extracellular matrix and play a role in the regulation of skeletal muscle development, growth, and function. Proteoglycans contain a central core protein with covalently attached glycosaminoglycans, which are composed of repeating disaccharide units. Glycosaminoglycans include chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate. The different types of proteoglycans in skeletal muscle may have specific functions in muscle growth and development (Velleman, 2002).

2.1.3 Muscle fiber types

Muscle fibers can be classified into different types according to their color, contractile properties, energy supply, and structure. Muscle fibers can be classified into red or white depending on their myoglobin content; red muscle fibers have higher myoglobin content, and thus, appear red in color. Meat can be classified as red or white, according to the overall color, which is relative in proportion of red and white fibers within the muscle. In poultry (chicken and turkey), breast meat is composed mainly of white fibers and thus, are referred to as white meat, whereas thigh meat, which is composed mainly of red fibers, are referred to as red meat (Barbut, 2002). Red and white muscle fibers are adapted to oxidative and glycolytic metabolism, respectively. According to muscle contraction speed, muscle fibers are categorized into slow and fast twitch which respond slowly and quickly to stimulation, respectively, and exhibit the physiological characteristics of red and white muscles, respectively. Muscle fiber classification may further be categorized into three types: Type I (slow twitch oxidative), Type IIa (fast twitch oxidative), and Type IIb (fast twitch glycolytic) (Kinsman et al., 1994). Type I fibers (red fibers) have high myoglobin content which supplies oxygen for oxidative metabolism. These fibers have a relatively high content of mitochondria, which are the organelles responsible for aerobic respiration of cells, and hence, they can contract for longer time and thus, fatigue occurs slowly. However, the contraction speed of these fibers is slower compared to white fibers. Type IIa fibers (red fibers) have medium myoglobin content and fatigue occurs on a medium speed compared to type I and type IIb fibers. Type IIb

fibers (white fibers) have low myoglobin content and have glycolytic metabolism, which can occur in the presence or absence of oxygen. These fibers have a relatively low content of mitochondria. The contraction speed of these fibers is faster compared to red fibers, and thus fatigue occurs faster (Barbut, 2002). Chicken *pectoralis* muscle (breast) is composed of type IIb muscle fibers (Smith and Fletcher, 1992), and chicken *sartorius* muscle (thigh) is composed of mainly type I muscle fibers (Suzuki et al., 1985).

2.2 Conversion of muscle to meat

The conversion of muscle to meat involves a series of biochemical events which occur after animal slaughter. An important pathway in the conversion of muscle to meat is glycolysis. In the living tissue, glycolysis produces adenosine triphosphate (ATP) for cellular functions. In *post mortem* muscle, the tissues attempt to preserve ATP concentrations to maintain homeostasis and the exsanguination (blood removal) of the animal results in the lack of oxygen transport to the muscle. The lack of oxygen and the need for ATP causes anaerobic glycolysis of muscle glycogen stores. Hence, during *post mortem* metabolism, glycogen and ATP levels decline and lactic acid accumulates in the muscle (Valin et al., 1992; Tarté and Amundson, 2006). As a result of the build up of lactic acid, muscle pH decreases usually from 7.2 (physiological pH of meat muscle proteins) to an ultimate pH of 5.3 - 5.8 (Kerry et al., 2002). The reduction in pH gradually leads to reduced efficiency of the sarcoplasmic reticulum Ca^{2+} pump which causes Ca^{2+} ions to leak into the cytosol. Calcium binds to troponin-C and triggers muscle contraction which leads to the formation of actin-myosin

cross-bridges. However, when more than 60% of the ATP has been utilized, the cross-bridges are not released, which results in the onset of *rigor mortis*, causing stiffening of muscles (Kahn, 1975; Tarté and Amundson, 2006). In poultry, rigor onset usually occurs in 2 to 4 h. Some important factors which determines the texture of meat is the extent of contraction and resolution of muscle during rigor (Lyon and Buhr, 1999).

An important biochemical activity which takes place during *post mortem* is tenderization of meat. In *post mortem* muscle, the release of calcium also activates protease enzymes, which are naturally found in muscle tissues and play an important role in meat tenderization (Tarté and Amundson, 2006). Some of the key enzymes are calpains and cathepsins. There are primarily two forms of calpains, μ - and m-calpain, which are located around the myofibrils and are activated by calcium (Edmunds et al., 1991). In the pH range of 7.0 - 7.2, μ -calpains are active; however this enzyme still remains active at lower pH (5.5 - 5.8) (Koochmaraie et al., 1986). The activity of μ -calpains increases with decreasing pH and reduces with decreasing temperatures (25 - 5°C). At a sarcoplasmic Ca^{2+} concentration of $<110\mu\text{M}$, μ -calpains are active, while m-calpains require higher levels of calcium ($>300\mu\text{M}$) (Hopkins and Thompson, 2001; Tarté and Amundson, 2006). The most active proteases during *post mortem* myofibrillar protein degradation are μ -calpains. Initial proteolysis begins as early as 3 h *post mortem* in which muscle protein breakdown occurs. Some structural changes can occur which include degradation of cytoskeletal proteins (i.e. titin, nebulin, desmin, and tropomyosin) that eventually breaks the structure of Z-line;

however μ -calpains does not generally affect myosin and actin (Goll et al., 1983; Huff-Lonergan and Lonergan, 1999). The primary regulator of μ -calpain proteolytic activity is its inhibitor, calpastatin (Tarté and Amundson, 2006). This type of enzyme competes with other protein substrates for proteolysis catalyzed by μ -calpain, thus inhibiting the hydrolysis of myofibrillar proteins by μ -calpain. Cathepsins are a group of acidic proteases which are located in the lysosomes (Xiong, 2004). They play a role in proteolysis which takes place at a later time *post mortem* during the aging cycle (>4 days) (Taylor et al., 1995). There are different types of cathepsins (i.e. cathepsins B, D, E, H, and L) that are capable of degrading similar types of proteins affected by calpains (i.e. titin and tropomyosin) (Ouali et al., 1987). Cathepsins can hydrolyze actin and myosin, however this generally does not occur at refrigerated temperatures. In order for cathepsins to be active, they require low pH for optimal activity (3.5 - 4.5) and they must be released from the lysosomal compartments (Ouali et al., 1987). Thus, it is generally believed that this type of enzymes has minimal involvement in meat *post mortem* meat tenderization (Huff-Lonergan and Lonergan, 1999; Xiong, 2004).

Another *post mortem* change which occurs is protein denaturation that results from the accumulation of lactic acid in the muscle, affecting meat quality. Due to the disruption of cell membranes resulting from myosin denaturation, drip loss occurs which causes light scattering on the meat surface; hence meat appears pale in color (Offer, 1991; Belitz et al., 2009). Generally, the ultimate pH of muscle depends on two factors: the rate (i.e. *post mortem* pH decline) and the

extent (i.e. muscle acidification) of *post mortem* glycolysis. Both of these factors are influenced by intrinsic factors (e.g. species, type of muscle, and variability between animals) and extrinsic factors (e.g. environmental temperatures) (Lawrie and Ledward, 2006).

2.3 Pale, soft, exudative (PSE) and dark, firm, dry (DFD) meat condition

2.3.1 PSE meat condition in pigs

The pale, soft, exudative (PSE) meat condition in pigs is primarily caused by an accelerated rate of *post mortem* glycolysis, which results from a rapid rate of pH decline at 45 min *post mortem* (pH below 5.8) and high carcass temperature (above 35°C) (Figure 2.2) (Honikel, 1987). The ultimate pH at 24 h *post mortem* (pH₂₄) of PSE meat is approximately 5.5 - 5.9 (Huff-Lonergan et al., 2003), which may be similar to that of normal meat. However, it is important to note that the combination of fast drop in pH and high muscle temperature during *post mortem* causes the development of PSE meat. In the pork industry, PSE meat refers to meat that has pale color, soft texture, and exudative surface. The combination of fast drop in pH with high muscle temperature causes protein denaturation in the muscle which results in poor meat quality (Santos et al., 1994; Cassens, 2000). The paleness of PSE meat is caused by the increased light scattering due to precipitation of sarcoplasmic proteins. In addition, there is increased drip loss due to the disintegration of cell membranes resulting from myosin denaturation, which leads to increased light scattering on the meat surface. PSE meat has a soft texture that which occurs from the exudates that fills the area between the muscle fiber bundles (Offer, 1991; Belitz et al., 2009).

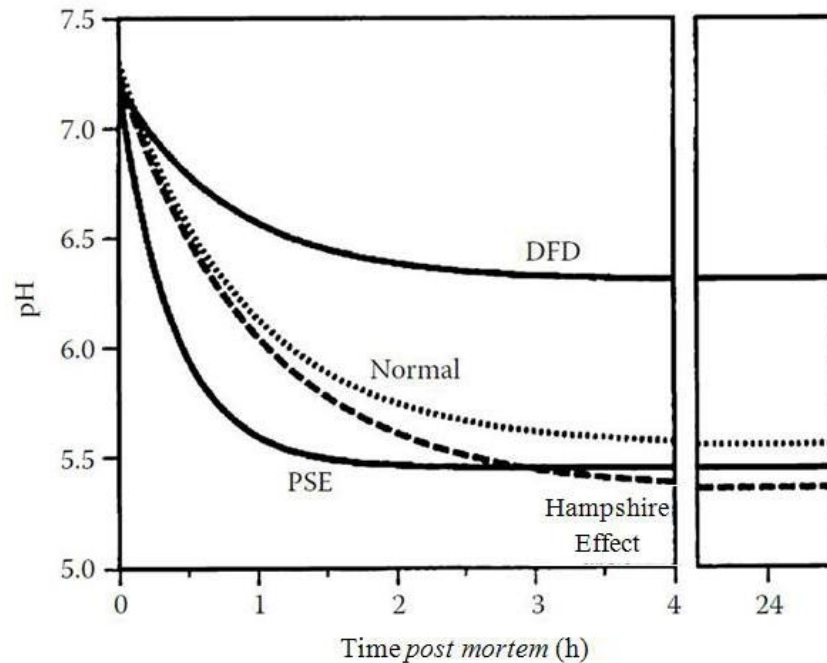


Figure 2.2. Effect of time *post mortem* on pH for several meat quality types. Reprinted from *QUALITY AND GRADING OF CARCASSES OF MEAT ANIMALS* by S. D. Morgan Jones. Copyright 1995 by CRC Press. Reproduced with permission of CRC Press in the format of Dissertation via Copyright Clearance Center.

There are numerous factors which have been investigated as possible causes for the abnormal *post mortem* glycolysis in PSE muscle, such as stress prior to slaughter. PSE meat condition is more associated with short-term (acute) pre-slaughter stress (Guidi and Castigliego, 2010). When pigs suffer from acute stress prior to slaughter, there will be rapid accumulation of lactic acid during early *post mortem* period due to the presence of sufficient muscle glycogen. This ultimately leads to muscle pH to values lower than 6.0 prior to the first hour of slaughter (D'Souza et al., 1998). In pigs, two genetic mutations play a major role in the development of PSE condition: Halothane (*Hal*) gene mutation (causing Porcine stress syndrome (PSS)) and Rendement Napole (*RN*) gene mutation

(Bowker et al., 2000). Pigs with porcine stress syndrome (PSS) are more prone to PSE development than normal pigs, mainly due to increased susceptibility to pre-slaughter stress. The PSS condition is linked to a single autosomal recessive gene, which is commonly referred to as the halothane (*Hal*) gene because diagnosis of the mutation can be made by exposure to halothane anesthesia (Rasmusen & Christian, 1976; Bowker et al., 2000). The presence of this gene results in a point mutation in the 615th amino acid (Arg615 to Cys615 substitution) of the sarcoplasmic reticulum Ca²⁺ release channel protein (ryanodine receptor (RYR)) (Fujii et al., 1991). In pigs with this mutation (*Hal*⁺), Ca²⁺ is released from the sarcoplasmic reticulum at twice the rate of release in normal pigs. Hence, the increased levels of Ca²⁺ in the muscle accelerate the rate of glycolysis which causes an accumulation of lactic acid during early *post mortem* period (Briskey, 1964; Cheah & Cheah, 1976; Mickelson et al., 1989). In stressed PSS susceptible pigs, malignant hyperthermia (MH) can occur, which causes elevated body temperatures. Regulation of cellular Ca²⁺ concentrations during early *post mortem* via the ryanodine receptor is one of the mechanisms through which PSE development may occur. PSE may also develop in the absence of the *Hal* gene (*Hal*⁻). *Hal*⁺ pigs have a lower Ca²⁺ sequestering ability of the sarcoplasmic reticulum from muscle than that of *Hal*⁻ pigs (Küchenmeister et al., 1999). The incidence of PSE in *Hal*⁺ pigs is five times higher than *Hal*⁻ pigs (Eikelenboom and Nanni-Costa, 1988). However, more research is needed to determine the role of Ca²⁺ metabolism in the PSE development of *Hal*⁻ pigs.

The Rendement Napole (*RN*) gene exists as two alleles: one dominant

(*RN*) and one recessive (*rn*⁺). Pigs with a mutation in the *RN* gene have elevated muscle glycogen levels, which lead to extended *post mortem* glycolysis resulting in meat with low ultimate pH. In particular, this has been observed in populations of pigs within the Hampshire breed, in which the animals have high levels of muscle glycogen at the time of slaughter. Hampshire pigs have a normal glycolytic rate; however they have an extended *post mortem* glycolysis which causes low ultimate pH (Monin et al., 1987). As a result, this leads to the production of “acid meat” and this genetic defect is often referred to as the “Hampshire Effect” (Figure 2.2) (Bowker et al., 2000). In *RN* carrier pigs, white muscle fibers may have up to 70% more glycogen in the sarcoplasmic and lysosomal compartments than normal pigs (Estrade et al., 1993; Bowker et al., 2000). Lundström et al. (1996) observed that pigs with this mutation have higher glycolytic potentials compared to *rn*⁺ pigs. Glycolytic potential is a method to predict lactate formation of muscle tissue at the time of slaughter by measuring the amount of glucose, glucose-6-phosphate, glycogen, and lactate (Monin et al., 1981). However, high levels of muscle glycogen may not always result in extended glycolysis which leads to low ultimate muscle pH (Bowker et al., 2000). In fact, Van Laack and Kauffman (1999) reported that the glycolytic potential in pork muscle accounts for approximately 49% of the variation in ultimate pH. This suggests that other factors, such as glycolytic enzymes may be involved in *post mortem* metabolism which could lead to low pH₂₄. Therefore, these studies showed that genetic mutations in swine are key factors that can lead to the development of PSE meat.

2.3.2 DFD meat condition in pigs

The dark, firm, dry (DFD) meat condition in pigs is associated with long-term (chronic) pre-slaughter stress. The DFD meat condition occurs when animals suffer from chronic stress prior to slaughter, which leads to muscle glycogen depletion, and thus prevents sufficient lactic acid from reaching muscle pH values lower than 6.0 at 24 h *post mortem* (Figure 2.2). This results in meat with higher ultimate pH than normal meat (Enfält et al., 1993; D'Souza et al., 1998). Pre-slaughter factors which could influence the development of DFD meat are season, animal handling, stocking density during transportation, lairage time, and on-farm fasting (Guárdia et al., 2005). Higher incidences of DFD meat were found in the winter season than in the summer (Galwey and Tarrant, 1978). Low temperatures deplete muscle glycogen concentrations when the pigs keep themselves warm, so they tend to become fatigued faster. Stress can arise from poor handling of animals, such as hitting and kicking the animals to facilitate movement and forcing them into transporting vehicles (Adzitey and Nurul, 2011). The stocking density during transportation is an important aspect which can result in stressful conditions. When a stocking density above 0.35 m² per 100 kg pig is available during transport, pigs are susceptible to stress due to trampling and/or fighting between pigs (Gade and Christensen, 1998). As a consequence, this leads to muscular fatigue and glycogen depletion. Guárdia et al. (2005) observed that the risk of developing DFD meat reduced by 11% when the stocking density decreased from 0.5 m² - 0.37 m² per 100 kg pig. After transportation, pigs are kept in lairage before slaughter. Longer lairage times were reported to be associated

with the increased development of DFD meat due to fighting of animals and resulting in bruises and skin blemishes (Warriss, 2003). An important aspect during lairage is the fasting times. A fasting time of 18 h between a pig's last feed and its slaughter has been recommended (Warriss, 1994). However, longer fasting times can increase the risk of developing DFD meat. During fasting, glycogen levels in the liver and muscles decreases leading to higher ultimate muscle pH (Warriss et al., 1989). DFD meat has shorter shelf life. The major problem with this type of meat is its darker appearance which affects the color of processed products and consumer acceptability (Allen et al., 1997).

2.3.3 PSE-like meat condition in poultry

In the past decade, the poultry industry has focused on resolving the PSE pork-like condition in poultry, while the condition has been studied in the pork industry for the last three decades (Barbut et al., 2005). The commercial incidence of PSE-like meat in broilers and turkeys ranges from approximately 5 - 40% of the birds (Barbut, 1996; Owens et al., 2000; Woelfel et al., 2002). Factors which influence the development of PSE-like poultry meat include season, environment, heat stress, and pre- and post-slaughter handling practices (Owens et al., 2009).

2.3.3.1 Potential causes

2.3.3.1.1 Genetics

The genetic makeup of poultry is more complex than in swine. The ryanodine receptor (RYR) is a calcium release channel located in the sarcoplasmic reticulum (SR) in muscle cells. This receptor has four subunits and in skeletal muscles of mammalian species, these subunits are composed of the same isoform

(RYR1) (Chiang et al., 2007). In avian species, there are two ryanodine receptor isoforms: α -RYR (most similar in primary structure and function to RYR1) and β -RYR (similar to mammalian RYR 3 isoform), in which both are expressed nearly in equal abundance in muscle cells (Ottini et al., 1996). The ryanodine receptor plays a major role in the regulation of Ca^{2+} and changes in its activity may have important implications for the development of PSE-like meat. In general, the regulation of calcium in muscles is dependent upon the process of excitation-contraction coupling, which follows a series of events. In avian muscles, depolarization is triggered by motor neurons that transmit a voltage across the sarcolemma. The dihydropyridine receptor (DHPR) is a protein located within the t-tubules of muscle fibers and is physically coupled to α -RYR. The depolarization induces a conformational change in the DHPR which causes the release of calcium from the SR into the sarcoplasm by α -RYR. Hence, increased local concentration of Ca^{2+} leads to the opening of β -RYR, which is peripherally located to t-tubules and in the junction of the SR. The increase in calcium then triggers muscle contraction. During muscle relaxation, Ca^{2+} is sequestered back to the SR via an ATP-dependent Ca^{2+} pump, preventing calcium from inducing muscle contraction (Strasburg and Chiang, 2009; Ziober et al., 2010).

Strasburg and Chiang (2009) suggested that changes in calcium channel activity by defects in either ryanodine receptor isoform or both isoforms may cause alterations in calcium regulation, which may ultimately lead to the development of PSE-like meat. According to these authors, the defects may be related to 1) mutation in the ryanodine receptor leading to calcium leak, 2)

alternative splicing of the channel protein resulting in proteins with changes in calcium release properties, 3) altered post-translational modification of the ryanodine receptor leading to changes in expression, or 4) abundance of channel proteins. Several researchers have investigated isoforms of ryanodine receptor at the molecular level and have discovered alternative transcript spliced variants in α -RYR isoform in poultry. Different transcript variants in turkey RYR were reported, which have resulted in deletions of base pairs leading to deletions of amino acid residues at different locations (Chiang et al., 2007; Strasburg and Chiang, 2009). It was hypothesized that these transcript variants could lead to a non-functional ryanodine receptor protein, which could then result in abnormal calcium regulation in the muscle. Hence, *post mortem* metabolism could then be altered. Factors regulating the expression of these variants are not clear, although Strasburg and Chiang (2009) hypothesized that heat stress may be a factor. Although these studies showed the identification of alternatively spliced transcript variants in α -RYR, splice variants in β -RYR still remains to be investigated. More research is needed to determine the relationship between genetics and alternative splicing activity as it may have important implications in the development of PSE-like condition in poultry.

2.3.3.1.1.1 Rapid growth

There has been an increase in consumer demand for poultry products over the past few decades, which have led to increased poultry production (Poultry Marketplace, 2010). Recent genetic selection of chickens and turkeys has contributed to fast growing birds, achieved in part, by enhancements in growth

rate and carcass yield. Currently, birds are marketed at approximately half the time and twice the body weight (BW) compared to 50 years ago (Barbut et al., 2008). However, it has been shown that faster growing or heavier birds are more susceptible to heat stress, as indicated by increased metabolic heat production and body temperatures (Lin et al., 2006). Updike et al. (2005) observed that the modern commercial turkeys selected for increased growth and breast muscle yield may be associated with decreased protein functionality, such as decreased water holding capacity and gel strength. Faster growing birds may also exhibit a greater incidence of muscle abnormalities. Swatland (1990) measured the growth of connective tissue in turkey breast of commercial fast growing turkeys, and at 15 weeks of age, the turkeys had approximately 35-fold increase in cross-sectional areas of muscle fibers compared with 1 week of age. It was found that the endomysium doubled in width, while perimysium increased 5-fold in width, which indicates that the connective tissues are not grown proportionally to muscle fibers. Hence, muscle fiber proportions and the amount of extracellular matrix space are altered and thus, affect the amount of proteoglycans present. Proteoglycans may be involved with the spacing of developing muscle fibers due to its ability to ionically associate with water. Hence, proteoglycans may be of importance in determining the water holding capacity of muscles (Velleman, 2002). Thus, selection of birds with faster growth may have direct effects on meat quality through changes in muscle structure.

2.3.3.1.2 Environmental causes

2.3.3.1.2.1 Antemortem stress factors

Antemortem stress factors that influence the development of PSE-like condition in poultry include environmental temperatures, transportation, and pre-slaughter handling practices. The incidence of PSE-like condition in turkeys is higher during the summer season when environmental temperatures are high. At high temperatures, the primary mechanism for heat loss (e.g. evaporative cooling) is impeded, thereby decreasing heat dissipation in birds resulting in stress (Yahav et al., 1995). McCurdy et al. (1996) reported the highest lightness (L^*) values in turkey breast meat indicating paler color during the summer season and the lowest in the winter season. McKee and Sams (1997) reported that chronically heat-stressed turkeys exhibited lower muscle pH, higher L^* values, and higher drip and cook loss as compared with control turkeys (non-heat-stressed). Petracchi et al. (2004) evaluated the effect of season on color variation in a large population of broiler breast meat (6,997) and reported higher L^* values in samples collected during the summer season compared to the ones collected during the winter, and it was found that paler breast meat was associated with low ultimate pH and reduced water holding capacity. Wang et al. (2009) conducted a study to determine the effects of heat stress on protein oxidation and functionalities of broiler breast meat and reported that when birds were exposed to heat for longer duration, protein functionality was affected. It was found that when birds were kept at 41°C for 5 h, there was a decrease in pH at 24 h *post mortem*, protein solubility, and gel strength. Increase in drip loss, cooking loss, and protein oxidation were also reported. Thus, pre-slaughter exposure to heat reduces the oxidative stability of broiler muscle proteins, which in turn affects protein functionality. Transportation

stress factors which are associated with meat quality problems include vibration and noise, heat stress, relative humidity, crowding, and air flow. Generally, research in swine has shown that transportation can lead to increased *post mortem* metabolism, which can affect meat quality. However, results were not conclusive on studies related to transportation stress and meat quality in poultry, which either showed an improvement, no change, or decrease in meat quality (Kannan et al., 1998; Owens and Sams, 2000; Debut et al., 2003). This may be due to the different conditions used in these studies, including transportation time, environmental temperatures, processing methods, and stocking density. Therefore, it is important for processors to be aware of transportation conditions that may lead to stressful situations for the birds. The primary causes of stress during pre-slaughter handling practices are physical stressors, such as crowding during catching and holding before slaughter (Owens et al., 2009). These physical stressors can create an environment with elevated heat released from the birds, which can further stress the animals.

2.3.3.1.2.2 Processing factors

The PSE-like meat condition may develop in normal, non-stressed birds if they are improperly processed. One of the primary processing conditions which can lead to the development of PSE-like meat is *post mortem* chilling. In poultry, turkeys are more susceptible to improper chilling due to their large body size and muscle mass (Owens et al., 2009). Low muscle pH at high body temperature causes more severe damage than muscles with the same pH at cooler temperatures. McKee and Sams (1998) observed that lower pH in turkey breast

meat combined with elevated *post mortem* temperature of the muscles at 20 and 40°C (e.g. slower chilling rate) had more meat quality defects (increased drip loss and cook loss) than at lower temperatures at 0°C after 4 h *post mortem* (e.g. faster chilling rate). At the same *post mortem* period, turkey breasts held at 40°C had higher L* values compared to samples at 20 and 0°C indicating paler meat color. Alvarado and Sams (2002) recommended that the temperature of turkey breast meat should reach 25°C or lower by 60 min *post mortem* to prevent reduction in meat quality. Thus, elevated *post mortem* temperatures resulting from slower chilling rates may be a key contributor in the development of PSE-like meat characteristics. Rathgeber et al. (1999) evaluated the effects of rate of *post mortem* glycolysis and delayed chilling on turkey breast meat quality. Based on 15 min *post mortem* breast muscle pH, turkey carcasses were classified as rapid glycolyzing (RG) with pH \leq 5.80 or normal glycolyzing (NG) with pH $>$ 6.00. It was found that delayed chilling of carcasses (110 min *post mortem*) increased breast meat L* values, decreased ultimate pH and cook yield, and had lower amounts of extractable proteins compared to the samples that was chilled at 20 min *post mortem*. It was also found that regardless of the rate of *post mortem* glycolysis, delayed chilling leads to defects in meat quality. Alvarado and Sams (2004) reported that slower chilling rates have contributed to PSE-like characteristics (decrease in pH and increase in cook loss); however, there was no effect on protein solubility. Thus, these studies showed that it is possible to artificially generate PSE-like meat under different processing conditions. Therefore, it is important for processors to take into consideration of chilling

conditions so as to prevent meat quality defects associated with PSE-like condition.

2.3.3.2 Potential solutions

Potential solutions to reduce or eliminate the PSE-like meat problem can be divided into short- and long-term approaches. Short-term approaches include minimizing the factors that can lead to the development of PSE-like meat in susceptible birds, which can be achieved through stress and heat reduction, and controlling chilling rate of meat. Long-term approaches include extensive research on genetics and selection, and utilization of alternative technologies to reduce or eliminate the PSE problem (Barbut et al., 2008).

2.3.4 DFD meat condition in poultry

The DFD meat condition in poultry is similar to the one observed in pigs. In poultry, this condition is associated with long-term pre-slaughter stress which causes glycogen depletion in the muscle. Consequently, the birds have less muscle glycogen stores at the time of slaughter to convert to lactic acid, and thus resulting in high ultimate muscle pH (Owens and Sams, 2000). Mallia et al. (2000) have investigated the physical characteristics of DFD turkey breast meat and revealed that this type of meat had darker color as revealed by lower L* values, lower cooking loss, higher water holding capacity, and higher gel strength in comparison to normal meat. It has been shown that transportation effects are key factors leading to the development of DFD meat condition in poultry. Owens and Sams (2000) have investigated the influence of transportation on turkey breast meat quality and observed that turkeys that have been transported for 3 h before

processing had higher muscle pH and lower L* values in turkey breasts in comparison to ones from non-transported turkeys. However, there was no difference in drip loss and cook loss between turkey breasts from transported and non-transported turkeys. Birds exposed to cold environment before slaughter are more susceptible to stress. Dadgar et al. (2010) has reported that the exposure of broilers to temperatures below 0°C during transportation increased the incidence (8%) of DFD breast meat. DFD broiler breast meat had darker color as indicated by lower L* values and higher water holding capacity compared to normal breast meat (Dadgar et al., 2010). A recent study was conducted by Dadgar et al. (2011) to evaluate the effect of acute cold exposure during transportation and lairage on broiler breast meat quality. It was reported that when birds were exposed to cold temperatures below -14°C for 3 h, a high incidence (>57%) of DFD breast meat (pH > 6.1 and L* < 46) was observed. When the birds were kept for 2 h in lairage following exposure to temperature below -8°C, there was further increase in the incidence of DFD by 20%. Birds exposed to cold temperatures before slaughter resulted in meat with higher pH, darker color, and less cook loss (Dadgar et al. 2011). Hence, these studies revealed that the DFD condition in poultry resulted in meat having similar or better meat quality in relation to normal meat.

2.4 Functional properties of muscle proteins

PSE-like meat condition in poultry affects the functional properties of muscle proteins. Functional properties of proteins are the physicochemical properties which affect their behavior in foods during processing, storage, and consumption. The physicochemical properties greatly influence product texture,

appearance, cooking yield, palatability, and consumer acceptance. Generally, functional properties of proteins are classified into three categories based on protein interactions: protein-water interaction (i.e. solubility), protein-fat interaction (i.e. emulsification), and protein-protein interaction (i.e. gelation) (Smith, 2001). Factors which can affect functional properties in food products are the source of muscle protein used, type and concentration of non-meat ingredients used, type of processing equipment used, and processing conditions. Different formulations used in meat processing (i.e. pH, salt concentration, and protein content) and processing conditions (i.e. cooking temperature) can affect the biochemical properties of proteins, and thus causes changes to the functional properties of proteins (Smith, 2001). Another key factor which can affect protein functionality is frozen storage of muscle proteins. Most processed muscle foods have a combination of several protein functionalities. Some of the important functional properties in cooked meat products are water binding, fat binding, and gelation (Xiong, 2004).

2.4.1 Functional groups in proteins

2.4.1.1 Hydrophobic groups

One of the largest forces regulating protein structure is the hydrophobic effect. In an aqueous medium, hydrophobic interactions are mainly driven by interactions with the solvent than interactions between the nonpolar groups (Janson and Rydén, 1998). Water molecules reorient themselves around the hydrophobic groups, which cause aggregation of nonpolar groups and thus, minimize their contact with water (Pratt and Cornely, 2004). Most hydrophobic

amino acids are buried in the interior of proteins and this arrangement stabilizes the protein structure. When proteins unfold, hydrophobic amino acid side chains would be exposed to the solvent. Hence, exposed hydrophobic groups on the protein surface may play a role in protein-protein interaction (i.e. hydrophobic interaction), such as protein aggregation, leading to the formation of protein gel upon heating (Kinsella and Whitehead, 1989).

The hydrophobicity of proteins can be grouped into two categories: average and surface hydrophobicity. The average hydrophobicity refers to the nonpolar character of amino acids within proteins, and is calculated by dividing the total hydrophobicity by the total number of amino acid residues. Surface hydrophobicity refers to the extent of distribution of hydrophobic residues on the protein surface (Damodaran, 1996; Zayas, 1997). Protein denaturation causes conformational changes, which in turn leads to the exposure of hydrophobic groups on the protein surface (LeBlanc and LeBlanc, 1992). Hence, increased exposure of hydrophobic groups on the protein surface can affect the surface activity of proteins by influencing the proteins' ability to migrate, adsorb, unfold, and form a layer at the interface (i.e. oil or air), and thus it affects its ability to participate in functional properties, such as emulsifying and foaming properties. Proteins with high surface hydrophobicity are able to readily adsorb at the interface between oil or air and water and form films around the oil droplets or air, and thus reducing interfacial or surface tension between the interfaces. This facilitates the formation of emulsions by preventing coalescence of oil droplets, and also facilitates the formation of foams (Kato et al., 1983).

2.4.1.2 Sulfhydryl groups

One of the most reactive amino acid side chains of proteins are the sulfhydryl groups (-SH) of cysteine residues. Sulfhydryl groups are highly susceptible to oxidation in the presence of iron salts or by other mild oxidizing agents, producing a disulfide bond (-S-S-) between two cysteine amino acid residues (Egorov, 1997). The changes in total (T-SH) and reactive (R-SH) sulfhydryl group contents reveal information on protein conformational changes and disulfide bond formation. Changes in protein conformation may lead to the exposure of reactive sulfhydryl groups. Hence, an increase in R-SH contents may reveal protein unfolding, while a decrease in T-SH contents may reveal the formation of disulfide bonds either within polypeptides or between polypeptides (Ko et al., 2007; Xia et al., 2009). The formation of disulfide cross-linking plays an important role in protein functionality, especially in heat induced gelation of proteins. Cross-linking in proteins can occur through oxidation of sulfhydryl groups and/or sulfhydryl-disulfide interchange reactions (Shimada and Cheftel, 1989). The formation of desirable textures in food is largely dependent on the ability of muscle proteins to form a gel. Gelation can be achieved through the cross-linking of proteins under heat treatment, which allows the establishment of a three-dimensional gel network with entrapment of water (Beveridge et al., 1984; Gerrard, 2002).

2.4.2 Protein functionality

2.4.2.1 Solubility

Protein solubility is one of the important functionalities of protein with profound effect in meat processing because it is an indicator of protein denaturation and it is related to other functional properties, such as emulsification, gelation, and water holding capacity (Zayas, 1997; Van Laack et al., 2000). In meat systems, functional properties of proteins are largely governed by their interactions with water. Hence, solubility of proteins refers to the amount of protein in a sample that is in solution relative to the total amount of protein present (Pelegri and Gasparetto, 2005). Protein solubility also relates to the balance between surface hydrophilic (protein-solvent) and hydrophobic (protein-protein) interactions with water. Thus, the relative proportion and distribution of surface hydrophobic and hydrophilic groups in proteins is important because it determines the degree of solvation by water (Vojdani, 1996). Intrinsic factors that can affect protein solubility are amino acid composition and sequence, molecular weight, and conformation and content of polar and nonpolar groups in proteins. Extrinsic factors can also affect protein solubility, which include pH, ionic strength, type of solvent, and processing conditions (Zayas, 1997).

The pH of a solution affects the distribution of net charge of proteins. At the isoelectric point (pI) of muscle proteins, which is usually pH 5.3 (Offer and Knight, 1988), proteins have a net zero charge (i.e. equal number of positive and negative charges) and are dominated by attractive forces. Hence, proteins tend to associate more closely, resulting in aggregation and precipitation. This type of protein-protein interaction in an aqueous medium is driven by hydrophobic interactions, which exists between nonpolar groups in proteins (Zayas, 1997).

When pH values are below and above the pI, proteins have a net positive or negative charge, respectively. In these acidic or basic conditions, there is an excess charge of the same sign, which leads to electrostatic repulsion among proteins, and consequently, leads to more protein-solvent interactions (Vojdani, 1996). There is increased solubility when electrostatic repulsion between protein molecules is greater than hydrophobic interactions. When solubility is plotted against pH, a U-shaped curve exists for most proteins (Choi and Park, 2002).

The addition of salt can also affect protein solubility. At low ionic strength (0.5 - 1.0 M), protein solubility increases with salt concentration and this is referred to as “salting in”. Salt ions bind to charged protein groups and thus increase solubility by electrostatic repulsion. “Salting-out” occurs at high salt concentration (greater than 1.0 M), where salt ions compete with protein for water, which causes protein precipitation (Zayas, 1997; Puri, 2006).

2.4.2.2 Water holding capacity

Water holding capacity is important in meat processing because it affects many of the physical properties of meat products, such as color, texture, juiciness, and tenderness. This ultimately will affect the overall product appearance, palatability, and yield. Water holding capacity is the ability of meat to hold water under applied force (i.e. heat, pressure) (Brewer, 2004). Generally, water is held by muscle proteins and is located within the interfilament spaces of thick and thin filaments of myofibrils. Muscle tissue contains approximately 75% water, of which they can exist in three forms: bound, immobilized or free. Approximately 4 - 5% of the water is in the bound form, which means that charged hydrophilic

groups on muscle proteins attract water, and thus form a tightly bound layer (Aberle et al., 2001). This bound water has reduced mobility and is not readily moved to other compartments in the muscle. Hence, there are minimal changes to this bound water in *post mortem* muscle (Offer and Knight, 1988). Freezing has little effect to this type of water. In the immobilized layer (also referred to as entrapped water), water is mainly held by attraction to the bound water and there is less orderly molecular orientation of water toward the charged groups. In early *post mortem* muscle, this water does not flow easily from the tissue, however it can turn to ice during freezing. This type of water is mainly affected during the conversion of muscle to meat when muscle acidification occurs (Huff-Lonergan, 2010). When the pH of muscle falls, this water becomes free water which can be easily lost as drip (Offer and Knight, 1988). In the free form, water is held only by weak capillary forces (Aberle et al., 2001). Some of the factors that can affect water holding capacity are pH, salt concentration, and processing conditions.

The pH of meat has profound influence on the water holding capacity. At *post mortem*, the accumulation of lactic acid causes a decline in pH from approximately 7.2 (physiological pH of meat muscle proteins) to 5.3 - 5.8. As the ultimate pH reaches near the isoelectric point of proteins (i.e. as in PSE meat), there is a reduction of electrostatic repulsion between myofilaments which reduces the space of myofilament lattice to hold water, thereby reducing WHC (Kerry et al., 2002). At the pI, water holding capacity is at the minimum as a result of decreased electrostatic repulsion between protein molecules, which further reduce the myofilament space to hold water and hence less water is

immobilized within the myofibrils. Consequently, this water is eventually lost as drip loss (Honikel, 2009).

Some of the methods to improve water holding capacity during processing are pH adjustment and addition of ingredients (i.e. salt, phosphates, and starches). As pH is increased during processing, proteins are more negatively charged, which results in an increase in repulsive forces. Thus, this causes swelling of myofibrils and more water is able to interact with proteins (Puolanne et al., 2001). Salt plays an important role in water retention properties of meat. Salt aids in the unfolding of myofibrillar proteins due to electrostatic repulsion of chloride ions and helps in solubilization. This causes an increase in interfilament space between the myofibrils where water can be entrapped. In addition, the charged groups in proteins become exposed where water can be bound (Rust, 1987). Phosphates can also be added in meat products to improve water holding capacity by increasing pH and ionic strength. Phosphates can also work in the presence of salt to aid in extraction of myofibrillar proteins (Claus et al., 1994). The addition of starch can be used to enhance water retention properties in meat. Zhang and Barbut (2005) have conducted a study to evaluate the effects of starch addition (potato and tapioca starch) on cooked PSE-like, normal, and DFD chicken breast meat batters and found that addition of starch partially enhanced water holding capacity as revealed by cooking loss.

2.5 High pressure processing (HPP) on meat proteins

Recently, food processors have adapted to changing consumer trends to meet their demands towards producing products that are more natural, minimally

processed, additive-free, and shelf stable (Yaldagard et al., 2008). High pressure processing (HPP) is an alternative technology to thermal processing, which has minimal effect on nutrients and is useful in food processing and preservation (Yaldagard et al., 2008). The first reported use of high pressure processing was on milk in 1899 by Hite to achieve a reduction in the microorganisms (Van Loey and Hendrickx, 2002). High pressure can affect microbial inactivation, enzymatic reactions, and structural and/or functional properties of foods which include protein denaturation, gel formation, and textural changes (Knorr, 1993; Dickinson and McClements, 1996).

The effects of high pressure are based on the principle of Le Chatelier. According to this principle, any change (i.e. phase transition, chemical reaction, or change in molecular configuration) that causes a reduction in volume will be enhanced by an increase in pressure and vice versa (Dickinson and McClements, 1996; Messens and Camp, 2003). Therefore, application of pressure favours any change that result in decrease in volume. The formation of hydrogen bonds is associated with reduction in volume and hence, pressure tends to stabilise their formation. Ionic bonds and hydrophobic interactions are generally broken down under pressure, while covalent bonds tend to be unaffected by pressure (Goodband, 2002).

2.5.1 High pressure effects on protein structure

The primary structure of proteins is made up of the sequence of amino acids, which is mainly joined together by peptide bonds (Campbell and Farrell, 2009). High pressure processing of food will not disrupt covalent bonds since

breakage of these bonds require very high energy of 213.1 kJ/mol; high pressure of 10,000 MPa can provide only 8.37 kJ/mol (Pauling, 1960; Morild, 1981).

The secondary structure of proteins refers to the arrangement of α -helix and β -sheet in the polypeptide chain, which is mainly formed by hydrogen bonds. These types of bonds are mainly formed by an electrostatic attraction between the hydrogen (partial positive charge) attached to an electronegative atom of a molecule (partial negative charge) which are mainly fluorine, nitrogen, or oxygen. The hydrogen bonds are the strongest intermolecular bonds (Beavon and Jarvis, 2003). The α -helix and β -sheet is stabilized by intramolecular and intermolecular hydrogen bonds, respectively (Heremans, 2002). β -sheet are more resistant to compressibility than α -helix under pressure, hence they form more stable structures (Tauscher, 1995). Pressure can promote the formation of intermolecular hydrogen bonds with water due to pressure-induced hydration while reducing the number of intramolecular hydrogen bond, resulting in conformational fluctuations of proteins (Cioni and Strambini, 1994). Pressure can also shorten the lengths of hydrogen bonds (Boonyaratanakornkit et al., 2002). Irreversible changes in the secondary structure can occur at pressure treatment above 300 MPa (Chapleau et al., 2003).

The tertiary structure of proteins is the spatial arrangement of amino acids in a three-dimensional structure within a polypeptide. Generally, this structure is stabilized by interactions which occur between amino acids, mainly by ionic interactions (between charged amino acid groups), hydrophobic interactions (hydrophobic groups tend to be clustered in the protein core and hydrophilic

groups tend to occur at the protein surface), hydrogen bonds and Van Der Waals forces (Buxbaum, 2007). In proteins, ion pairs are short-range, attractive interactions which occur between negatively and positively charged amino acid side chains. Salt bridges contain both hydrogen bonds as well as electrostatic interactions (Kuchel et al., 2009). The dissociation of ion pairs or disruption of salt bridges is favoured by high pressure because such a change depends on volume decrease by electrostriction, which is the contraction of solvents (i.e. water) due to the arrangement of polar solvent molecules surrounding the charged groups. Electrostriction may be reversible upon release of pressure (Chalikian et al., 1993; Tewari, 2007). Hydrophobic interactions are a major driving force for protein folding because they allow nonpolar amino acid side chains to cluster in the protein core (Gilbert, 2000). Pressure induces protein unfolding due to the disruption of hydrophobic bonds and salt bridges, which leads to the exposure of hydrophobic groups on the protein surface (Galazka et al., 2000; Boonyaratanakornkit et al., 2002). Van Der Waals forces are short-range, weak attractive interactions which occur between atoms and molecules (Gilbert, 2000). Generally, pressure favours Van Der Waals forces because these forces improve the packing density of proteins which decreases the volume. However, the dissociation of oligomeric proteins can occur when pressure is between 100 to 200 MPa, which results in the substitution of Van Der Waals forces between amino acid residues by protein-water interactions. The formation of amino acid-water interactions is more favourable to pressure than Van Der Waals forces

because they produce stronger and shorter bonds, which further decrease the volume (Silva, 1993; Jaenicke, 1998; Boonyaratanakornkit et al., 2002).

The quaternary structure of proteins is the association of two or more polypeptide chains to form a complex and is formed by the interaction of noncovalent bonds between the polypeptide chains. This structure is mainly stabilized by the same interactions found in the tertiary structure and hence, it is expected to have similar responses to pressure (Engelking, 2004).

Some of the extrinsic factors which can influence the modification of proteins are temperature, pH, ionic strength, protein structure and concentration, pressure level and time. When proteins are exposed to pressures below 100 to 200 MPa, there is reversible dissociation of subunits or partial unfolding. However, when pressures are above 200 MPa, changes are generally irreversible and may cause protein denaturation with unfolding of proteins or formation of aggregates (Chapleau and de Lamballerie-Anton, 2003). These changes seem to be related to the increased conformational fluctuations of amino acid side chains in proteins by the effect of pressure (Hummer et al., 1998; Tanaka et al. 2000). This generates pathways for water to penetrate into the core of proteins which gradually fills the cavities and cause swelling of proteins, leading to the opening of the protein structure.

2.5.2 High pressure induced protein gelation

Protein gelation is an important functional property in food systems because it provides mechanical integrity to food by formation of a gel network, and thus, it affects textural and rheological properties (Franks, 1988). The

different mechanisms of protein gel formation can be classified into physically induced (heat and pressure) and chemically induced (acid, ionic, and enzymatic) gelation reactions. A protein gel is a three-dimensional network of cross-linked protein chains which exists as an intermediate form between solid and liquid phase (Venugopal, 2006). Food protein gels are classified in different ways. According to the supramolecular structure, they may be either true cross-linked polymer networks or particle gels consisting of strands or clusters of aggregated protein. Gels can be thermoreversible, which means that they can melt upon heating and gel upon cooling. Gels can also be thermoset, which means that once they are formed by heating, they will not melt. According to the physical properties, gels can be brittle or ductile and/or opaque or translucent (Aguilera et al., 2004). Protein gelation refers to the transformation of a protein from the “sol” state to a “gel-like” state that leads to the formation of a network structure (Fennema, 1996).

High pressure processing can be used to induce gelation of muscle proteins. By using this technology, analogues of certain food products can be produced and novel textures can also be created (Messens et al., 1997). The basic mechanisms of protein gelation differ to some extent in pressure induced compared to heat induced gelation. However, it is important to understand the mechanisms in heat induced gelation, because high pressure induced gelation is commonly accomplished in addition to heat. Heat induced gelation is a common method to obtain gels, since heat treatment is generally used in the preparation and processing of foods. Heat induced gelation is a two-step process. The first

step is protein denaturation, which involves unfolding or dissociation of the protein molecule. The second step involves the association and aggregation of unfolded molecules forming higher molecular weight complexes through hydrophobic interactions which are weak attractive interactions between hydrophobic groups. This results in a three-dimensional network of aggregates or strands of molecules cross-linked by noncovalent bonds or less frequently, by covalent bonds (i.e. disulfide bonds) that is capable of holding water (Totosaus et al., 2002). The first step in gelation may be reversible and the second step is usually irreversible. Generally, gelation happens if one or more of the following conditions are met: (a) protein concentration is above a critical value, (b) partial denaturation of protein, or (c) environmental conditions are adequate (Aguilera et al., 2004).

Pressure treatment can affect proteins, undergoing reversible or irreversible structural modifications. High pressure can affect molecular interactions and protein conformation, which can lead to protein denaturation, aggregation, or gelation (Messens et al., 1997). According to Hawley's theory, there are two distinct states of the protein (native and denatured). A general pressure-temperature phase diagram for proteins shows these two states (Figure 2.3). In the phase diagram, proteins maintain its native structure under the elliptical shape area, whereas denaturation of proteins occurs outside this area. Hence, according to the phase diagram, the native and denatured state of proteins depends on different combinations of pressure (P) and temperature (T)

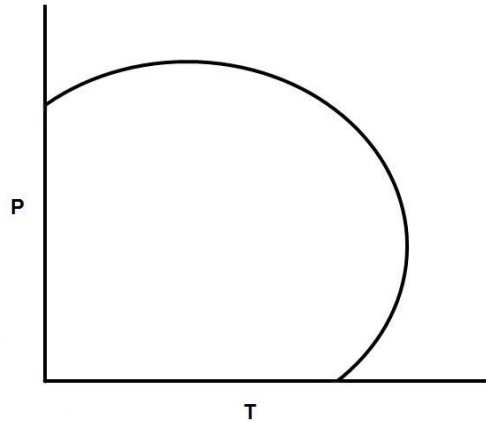


Figure 2.3. A general pressure-temperature phase diagram for proteins. P: pressure; T: temperature.

(Messens et al., 1997). Hawley's theory for the elliptical phase diagram is based on general assumptions that only two possible states of the protein exists. However, an intermediate state of proteins called "molten globule" was also found during protein denaturation (Ptitsyn and Uversky, 1994).

High pressure induced gelation depends on the protein system (i.e. type of protein, protein structure, and concentration) extrinsic factors (i.e. pH, ionic strength, and presence of other compounds) and on the processing conditions (i.e. pressure level, temperature, and time) (Colmenero, 2002). Generally, pressurizing above 100 to 150 MPa at low temperatures induces protein denaturation, thus favouring unfolding and solubilisation, which are necessary for the first stage of gelation. When pressure is released, the protein is restructured initially by hydrogen bonding, and subsequently, the protein is stabilized by hydrophobic or electrostatic forces. The refolding process establishes new interactions to produce protein aggregation, which are necessary for the formation of a gel network.

Pressures of 100 to 200 MPa can induce the formation of intermolecular disulfide bonds (Ko et al., 2003).

Protein solubilization is one of the important steps to take part in protein interactions such as gelation. Under pressure treatment, actomyosin depolymerize into actin and myosin (Cheftel and Culioli, 1997). This causes an increase in the solubility of myofibrillar proteins, thereby improving gelation properties. Macfarlane (1974) reported an increase in solubility of sheep myofibrillar proteins when subjected to pressurization at 150 MPa (0°C, 5 min). It was also reported that the effect of pressure on myofibril solubility was dependent on temperature, which resulted in more protein solubility at temperature above 0°C than at 30°C (Macfarlane and McKenzie, 1976). An increase in the solubility of chicken myofibrils was also reported when it was subjected to pressure treatment at 200 MPa for 10 to 20 min (Iwasaki et al., 2006). It was also found that pressure caused the dissociation of thick and thin filaments, which may be the reason for improved solubilization.

Pressure treatment before cooking increased the gel strength and elasticity in various muscle proteins. Ko (1996) observed improved gelation in milkfish paste which have been pressurized at 100, 300, and 500 MPa for 60 min at 0°C with subsequent heating at 90°C for 10 min. Similar results were reported in which pressurization at 200 MPa at 4°C for 60 min before heat treatment at 50°C for 60 min improved the gel forming ability of tilapia muscle proteins, compared to heat treated samples without pre-treatment of pressure (Hwang et al., 2007). An increase in the formation of disulfide bonds could be induced in the pressurized

gels during subsequent heating and thus, samples after cooking were more elastic and had the highest gel strength. Hsu and Jao (2007) observed that the structures of tilapia myosin were not affected by low pre-treatment of pressure at 50 MPa (4°C, 60 min). In this study, it was also found that a pressure pre-treatment at 200 MPa resulted in a gel with the highest values of breaking force in tilapia meat paste. Greater amount of hydrogen bonds was also found in gels with a 200 MPa pre-treatment, which may have contributed to improved gel forming ability. The conditions of low pressure level (200 MPa) or low temperature (3°C) may have favored the formation of hydrogen bonds (Pérez-Mateos et al., 1997). Ashie and Lanier (1999) found that pressure pre-treatment at 250 and 300 MPa at 4°C with subsequent cooking induced protein gelation in minced turkey breast paste. Similar results were reported by Iwasaki et al. (2006) in which an increase in elasticity of gels from chicken myofibrils was observed under a pressure pre-treatment at 200 MPa for 15 min with subsequent cooking at 70°C for 20 min. In this study, the microstructure of these gels revealed fine strand network, as opposed to heat induced gels which showed bundles of myofibrils. Generally, pressure induced gels of fish and chicken proteins were softer and glossier compared with heat induced gels (Okamoto et al., 1990). For example, cod myofibrillar protein formed a soft white gel when it was pressurized at 200 MPa (Angsupanich et al., 1999). Hence, it has been shown that high pressure processing is effective in improving the gel forming ability of different types of muscle proteins.

2.5.3 Enhancing water retention property using high pressure processing

The ability of gels to hold water is important because it affects the overall texture and palatability of the final product. The water holding properties are critical, especially in the formulation of meat products, where juiciness and tenderness of products are affected. Fernández-Martín et al. (1997) observed the improvement of water holding capacity of pork meat batters that were pressurized at 200 and 400 MPa and from 10 to 70°C (30 min). All pressurized samples showed very low exudation as revealed by less than 0.3% cook loss. The improvement of water holding capacity was also found in chicken breast meat batters. Fernandez et al. (1998) measured the weight loss in chicken meat batters which indicates the percentage of fluid released after heating at 20 to 22°C. It was found that at 200 and 400 MPa, weight loss in the chicken meat gels reduced by 44 and 78%, respectively. The amount of salt added in meat formulation is of particular interest because it affects the extraction of salt soluble proteins which influences the overall texture and cook yield of meat products. Generally, 1.8 to 2.0% of salt is added to meat formulations (Sikes et al. 2009). Pressure treatment at 150 MPa at 20°C for 5 min resulted in lower cook loss of frankfurters containing beef and pork with 1.5% salt compared to control (unpressurized) samples (Crehan et al., 2000). Similarly, Sikes et al. (2009) investigated the effects of pressure on reduction of cook loss in beef sausage batters with different salt contents. When no salt (0%) was added to the batters, samples pressurized at 200 MPa for 2 min at 10°C had the same level of cook loss (%) as the control (unpressurized) samples. However, when 0.5, 1.0 and 2.0% of salt were added to

the batters, there was reduction in cook loss. It was also found that pressure treated samples with 1.0% salt added resulted in similar cook loss values as control (unpressurized) samples containing 2.0% salt. Similar findings were reported by Iwasaki et al. (2006), where a reduction in cooking loss was found in pork meat patty containing 1.0% salt compared to non-pressure treated samples.

These studies have shown that pressure affects protein interactions which can improve protein functionality in different types of muscle proteins. The increase in protein solubilization can be achieved by using this technology, which can ultimately lead to improvements in water retention properties and protein gelation. However, the pressure effects on different meat systems depend on different combinations of pressure, time and temperature.

2.6 Research objectives and outline of thesis

The main objective of this thesis project was to characterize different properties of proteins in turkey breast meat with different ultimate pH at 24 h *post mortem* (pH₂₄) in fresh and frozen conditions and to improve the functional properties of pale, soft, exudative (PSE)-like turkey meat by application of high pressure processing (HPP). In order to accomplish this goal, different sub-objectives were defined:

- (a) Determine the biochemical and functional properties of proteins in turkey breast meat with different pH₂₄.
- (b) Assess the effect of frozen storage on the biochemical and functional properties of proteins in turkey breast meat with different pH₂₄.
- (c) Investigate the effect of pH at 24 h *post mortem* on rheological and textural properties on frozen turkey breast meat proteins.
- (d) Evaluate functional, textural, and rheological properties of proteins in turkey breast meat with low and normal pH that have been subjected to high pressure processing (HPP) and determine the optimal high pressure processing conditions to improve protein functionality.

The outline of the thesis is as follows:

Chapter 1 is the introduction to the research project.

Chapter 2 is a review of literature pertaining to the factors affecting protein functionality (PSE-like condition) and overview of high pressure processing.

Chapter 3 evaluates the effect of ultimate pH at 24 h *post mortem* on the biochemical and functional properties of proteins in turkey breast meat in fresh and frozen storage. Properties investigated include expressible moisture, calcium activated adenosine triphosphatase (Ca^{2+} -ATPase) enzyme activity, protein solubility, protein surface hydrophobicity, reactive (R-SH) and total (T-SH) sulfhydryl content, and protein carbonyl content. The results presented in this chapter have been accepted for publication in the journal of Food Chemistry.

Chapter 4 focuses on the functional, rheological, and textural properties of proteins in frozen turkey breast meat with different ultimate pH at 24 h *post mortem*. Properties investigated were emulsifying and foaming properties, cooking loss, sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) profile, gel strength, and textural and rheological properties. The results presented in this chapter have been submitted for publication in the journal of Poultry Science.

Chapter 5 attempts to identify the optimal high pressure processing conditions to improve the functional properties of PSE-like turkey meat. Properties investigated include protein solubility, protein surface hydrophobicity, reactive (R-SH) and total (T-SH) sulfhydryl content, expressible moisture, SDS-PAGE profile, textural and rheological properties. The results presented in this chapter have been submitted for publication in journal of Innovative Food Science and Emerging Technologies.

Chapter 6 and 7 are concluding chapters, where the project summary, industrial relevance and implications are described.

2.7 REFERENCES

- Aberle, E. D., J. C. Forrest, D. E. Gerrard, E. W. Mills, H. B. Hedrick, M. D. Judge, and R. A. Merkel. 2001. Principles of Meat Science. 4th ed. E. D. Aberle, J. C. Forrest, D. E. Gerrard, E. W. Mills, H. B. Hedrick, M. D. Judge, and R. A. Merkel, eds. Kendall Hunt Publishing Company, Dubuque, Iowa.
- Adzitey, F. and H. Nurul. 2011. Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences - A mini review. Int. Food Res. J. 18:11-20.
- Aguilera, J. M. 2004. Proteins in Food Processing. R. Y. Yada, ed. Woodhead Publishing Limited and CRC Press, Boca Raton, FL, USA.
- Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. Poult. Sci. 76:1042-1046.
- Alvarado, C. Z. and A. R. Sams. 2002. The role of carcass chilling rate in the development of pale, exudative turkey *pectoralis*. Poult. Sci. 81:1365-1370.
- Alvarado, C. Z. and A. R. Sams. 2004. Turkey carcass chilling and protein denaturation in the development of pale, soft, and exudative meat. Poult. Sci. 83:1039-1046.
- Alvarado, C. Z. and C. M. Owens. 2006. Poultry: Chemistry and biochemistry. Pages 1-31 in Handbook of Food Science, Technology and Engineering. Y. H. Hui, ed. CRC Press, Taylor & Francis, Boca Raton, FL, USA.

- Angsupanich, K., M. Edde, and D. A. Ledward. 1999. Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. *J. Agric. Food Chem.* 47:92-99.
- Ashie, I. N. A. and T. C. Lanier. 1999. High pressure effects on gelation of surimi and turkey breast muscle enhanced by microbial transglutaminase. *J. Food Sci.* 64:704:708.
- Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. *Can. J. Anim. Sci.* 76:455-457.
- Barbut, S. 2002. *Poultry Products Processing: An Industry Guide*. S. Barbut, ed. CRC Press, Boca Raton, FL, USA.
- Barbut, S., L. Zhang, and M. Marcone. 2005. Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poult. Sci.* 84:797-802.
- Barbut, S., A. A. Sosnicki, S. M. Lonergan, T. Knapp, D. C. Ciobanu, L. J. Gatcliffe, E. Huff-Lonergan, and E. W. Wilson. 2008. Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat Sci.* 79:46-63.
- Beavon, R. and A. Jarvis. 2003. Bonding and structure. Pages 31-46 in *Structure, Bonding, and Main Group Chemistry*. R. Beavon and A. Jarvis, eds. Nelson Thomas Ltd., UK.
- Belitz, H. D., W. Grosch, and P. Schieberle. 2009. Meat. Pages 563-616 in *Food Chemistry*. 4th ed. H. D. Belitz, W. Grosch, and P. Schieberle, eds. Springer-Verlag, Berlin, Heidelberg.

- Beveridge, T., L. Jones, and M. A. Tung. 1984. Progel and gel formation and reversibility of gelation of whey, soybean, and albumen protein gels. *J. Agric. Food Chem.* 32:307-313.
- Boonyaratanakornkit, B. B., C. B. Park, and D. S. Clark. 2002. Pressure effects on intra- and intermolecular interactions within proteins. *Bio. et Bio. Act.* 1595:235-249.
- Bowker, B. C., A. L. Grant, J. C. Forrest, and D. E. Gerrard. 2000. Muscle metabolism and PSE pork. *Proc. Am. Soc. Anim. Sci.* 1:8.
- Brewer, M. S. 2004. Water-holding capacity. Pages 242-249 in *Encyclopedia of Meat Sciences*. W. Jensen, C. Devine, and M. Dikeman, eds. Academic Press, UK.
- Briskey, E. J. 1964. Etiological status and associated studies of pale, soft, exudative porcine musculature. Pages 13:89-178 in *Advances in Food Research*. C. O. Chichester, ed. Academic Press Inc., USA.
- Buxbaum, E. 2007. Protein structure. Pages 13-37 in *Fundamentals of Protein Structure and Function*. E. Buxbaum, ed. Springer, USA.
- Campbell, M. K. and S. O. Farrell. 2009. The three-dimensional structure of proteins. Pages 87-122 in *Biochemistry*. 6th ed. M. K. Campbell and S. O. Farrell, eds. Thomson Brooks/Cole, USA.
- Cassens, R. G. 1994. Understanding muscle and meat. Pages 11-32 in *Meat Preservation: Preventing Losses and Assuring Safety*. R. G. Cassens, ed. Food & Nutrition Press, Inc., USA.

- Cassens, R. G. 2000. Historical perspectives and current aspects of pork meat quality in the USA. *Food Chem.* 69:357-363.
- Chalikian, T. V., A. P. Sarvazyan, and K. J. Breslauer. 1993. Partial molar volumes, expansibilities, and compressibilities of α,ω -aminocarboxylic acids in aqueous solutions between 18 and 55°C. *J. Phys. Chem.* 97:13017-13026.
- Chapleau, N. J. and M. I. de Lamballerie-Anton. 2003. Changes in myofibrillar proteins interactions and rheological properties induced by high-pressure processing. *Eur. Food Res. Technol.* 216:470-476.
- Chapleau, N., C. Mangavel, J. Compoint, and M. de Lamballerie-Anton. 2003. Effect of high-pressure processing on myofibrillar protein structure. *J. Sci. Food. Agric.* 84:66-74.
- Cheah, K. S. and A. M. Cheah. 1976. The trigger for PSE condition in stress-susceptible pigs. *J. Sci. Food Agric.* 27:1137-1144.
- Cheftel, J. C. and J. Culioli. 1997. Effects of high pressure on meat: A review. *Meat Sci.* 46:211-236.
- Chiang, W., H. Yoon, J. E. Linz, J. A. Airey, and G. M. Strasburg. 2007. Divergent mechanisms in generating molecular variations of α RYS and β RYS in turkey skeletal muscle. *J. Mus. Res. Cell. Motil.* 28:343-354.
- Choi, Y. J. and J. W. Park. 2002. Acid-aided protein recovery from enzyme-rich Pacific Whiting. *J. Food Sci.* 67:2962-2967.
- Cioni, P. and G. B. Strambini. 1994. Pressure effects of protein flexibility monomeric proteins. *J. Mol. Biol.* 242:281-301.

- Claus, J. R., J. Colby, and G. J. Flick. 1994. Processed Meats/Poultry/Seafood. Pages 106-113 in *Muscle Foods: Meat, poultry, and seafood technology*. D. M. Kinsman, A. W. Kotula, and B. C. Breidenstein, eds. Chapman & Hall, NY, USA.
- Colmenero, F. J. 2002. Muscle protein gelation by combined use of high pressure/temperature. *Trends Food Sci. Technol.* 13:22-30.
- Crehan, C. M., D. J. Troy, and D. J. Buckley. 2000. Effects of salt level and high hydrostatic pressure processing on frankfurters formulated with 1.5 and 2.5% salt. *Meat Sci.* 55:123-130.
- Dadgar, S., E. S. Lee, T. L. V. Leer, N. Burlinguette, H. L. Classen, T. G. Crowe, and P. J. Shand. 2010. Effect of microclimate temperature during transportation of broiler chickens on quality of the pectoralis major muscle. *Poult. Sci.* 89:1033-1041.
- Dadgar, S., E. S. Lee, T. L. V. Leer, T. G. Crowe, H. L. Classen, and P. J. Shand. 2011. Effect of acute cold exposure, age, sex, and lairage on broiler breast meat quality. *Poult. Sci.* 90:444-457.
- Damodaran, S. 1996. Functional properties. Pages 167-224 in *Food Proteins: Properties and Characterization*. S. Nakai and H. W. Modler, eds. Wiley-VCH, Inc., USA.
- Debut, M., C. Berri, E. Baéza, N. Sellier, C. Arnould, D. Guémené, N. Jehl, B. Boutten, Y. Jégo, C. Beaumont, and E. Le Bihan-Duval. 2003. Variation in chicken technological meat quality in relation to genotype and preslaughter stress conditions. *Poult. Sci.* 82:1829-1838.

- Dickinson, E. and D. J. McClements. 1996. Molecular basis of protein functionality. Pages 27-80 in *Advances in Food Colloids*. E. Dickinson and D. J. McClements, eds. Chapman & Hall, Great Britain.
- D'Souza, D. N., R. D. Warner, B. J. Leury, and F. R. Dunshea. 1998. The effect of dietary magnesium aspartate supplementation on pork quality. *J. Anim. Sci.* 76:104-109.
- Edmunds, T., P. A. Naganis, S. K. Sathe, V. F. Thompson, and D. E. Goll. 1991. Comparison of the autolyzed and unautolyzed forms of mu- and m-calpain from bovine skeletal muscle. *Biochim Biophys Acta.* 1077:197-208.
- Egorov, T. A. 1997. Identification of cysteine residues and disulfide bonds in proteins. Pages 259-268 in *Protein Structure Analysis, Preparation, Characterization, and Microsequencing*. R. M. Kamp, T. Choli-Papadopoulo, and B. Wittmann-Liebold, eds. Springer-Verlag, Berlin, Heidelberg.
- Eikelenboom, G. and L. Nanni-Costa. 1988. Fibre optic probe measurements in Landrace pigs of different halothane phenotypes. *Meat Sci.* 23:9-19.
- Enfält, A., K. Lundström, and U. Engstrand. 1993. Early post mortem pH decrease in porcine *M. Longissimus dorsi* of PSE, normal, and DFD quality. *Meat Sci.* 34:131-143.
- Engelking, L. R. 2004. Protein structure. Pages 18-23 in *Textbook of Veterinary Physiological Chemistry*. L. R. Engelking, ed. TetonMedia, USA.
- Estrade, M., X. Vignon, E. Rock, and G. Monin. 1993. Glycogen hyperaccumulation in white muscle fibres of RN⁻ carrier pigs. A biochemical and ultrastructural study. *Comp. Biochem. Physiol.* 104(B):321-326.

- Feiner, G. 2006. Meat Products Handbook: Practical Science and Technology. G. Feiner, ed. Woodhead Publishing Limited and CRC Press, Boca Raton, FL, USA.
- Fennema, O. R. 1996. Food Chemistry. 3rd ed. O. R. Fennema, ed. Marcel Dekker Inc., NY, USA.
- Fernández-Martín, F., P. Fernández, J. Carballo, and F. J. Colmenero. 1997. Pressure/heat combinations on pork meat batters: Protein thermal behavior and product rheological properties. *J. Agric. Food Chem.* 45:4440-4445.
- Fernandez, P., S. Cofrades, M. T. Solas, J. Carballo, and F. Jiménez-Colmenero. 1998. High pressure-cooking of chicken meat batters with starch, egg white, and iota carrageenan. *J. Food Sci.* 63:267-271.
- Franks, F. 1988. Functional attributes of protein isolates: Foods chester myers. Pages 491-546 in *Characterization of Proteins*. F. Franks, ed. The Humana Press, Inc., USA.
- Fujii, J., K. Otsu, F. Zorzato, S. de Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253:448-451.
- Gade, P. B. and L. Christensen. 1998. Effect of different stocking densities during transport on welfare and meat quality in Danish slaughter pigs. *Meat Sci.* 48:237-247.

- Galazka, V. B., E. Dickinson and D. A. Ledward. 2000. Influence of high pressure processing on protein solutions and emulsion. *C. Opin. Coll. Int. Sci.* 5:182-187.
- Galwey, W. J. and P. V. Tarrant. 1978. An investigation of high pH₂₄ pig meat in Irish bacon factories. *Irish J. Food Sci. Technol.* 2:21-30.
- Gerrard, J. A. 2002. Protein-protein crosslinking in food: Methods, consequences, and application. *Trends Food Sci. Technol.* 13:391-399.
- Gilbert, H. F. 2000. Protein structure. Pages 6-21 in *Basic Concepts in Biochemistry: A Student's Survival Guide*. H. F. Gilbert, ed. McGraw-Hill Companies, Inc., USA.
- Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Sgannon, S. K. Sathe, and M. Muguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. *J. Food Biochem.* 7:137-177.
- Goodband, R. 2002. Functional properties of fish proteins. Pages 73-80 in *Seafoods-Quality, Technology and Nutraceutical Applications*. C. Alasalvar and T. Taylor, eds. Springer-Verlag, Berlin Heidelberg.
- Guàrdia, M. D., J. Estany, S. Balasch, M. A. Oliver, M. Gispert, and A. Diestre. 2005. Risk assessment of DFD meat due to pre-slaughter conditions in pigs. *Meat Sci.* 70:709-716.
- Guidi, A. and L. Castigliego. 2010. Poultry meat color. Pages 359-388 in *Handbook of Poultry Science and Technology, Secondary Processing*. I. Guerrero-Legarreta, ed. John Wiley & Sons, Inc., USA.

- Heremans, K. 2002. The effects of high pressure on biomolecules. Pages 23-46 in Ultra High Pressure Treatments of Foods. M. E. G. Hendrickx and D. W. Knorr, eds. Kluwer Academic/Plenum Publishers, NY, USA.
- Honikel, K. O. 1987. Influence of chilling on meat quality attributes of fast glycolysing pork muscles. Pages 273-283 in Evaluation and Control of Meat Quality in Pigs. P. V. Tarrant, G. Eikelenboom, and G. Monin, eds. Martinus Nijhoff, Dordrecht, Netherlands.
- Honikel, K. O. 2009. Moisture and water-holding capacity. Pages 315-334 in Handbook of Muscle Foods Analysis. L. M. L. Nollet and F. Toldrá, eds. CRC Press, Taylor & Francis, Boca Raton, FL, USA.
- Hopkins, D. L. and J. M. Thompson. 2001. Inhibition of protease activity 2. Degradation of myofibrillar proteins, myofibril examination and determination of free calcium levels. *Meat Sci.* 59:199-209.
- Hsu, K. C. and C. L. Jao. 2007. Effect of pretreatment of hydrostatic pressure on physicochemical properties of Tilapia muscle protein gels induced by setting. *J. Food Sci.* 72:E73-E78.
- Huff-Lonergan, E. and S. M. Lonergan. 1999. Postmortem mechanisms of meat tenderization. Pages 229-251 in Quality Attributes of Muscle Foods. Y. L. Xiong, C. T. Ho, and F. Shahidi, eds. Kluwer Academic/Plenum Publishers, NY, USA.
- Huff-Lonergan, E., S. M. Lonergan, and D. H. Beermann. 2003. Animal growth and meat quality. Pages 220-232 in Biology of Growth of Domestic Animals. C. G. Scanes, ed. Iowa State Press, USA.

- Huff-Lonergan, E. 2010. Chemistry and biochemistry of meat. Pages 5-24 in Handbook of Meat Processing. F. Toldrá, ed. Blackwell Publishing, USA.
- Hummer, G., S. Garde, A. E. García, M. E. Paulaitis, and L. R. Pratt. 1998. The pressure dependence of hydrophobic interactions is consistent with the observed pressure denaturation of proteins. *Proc. Natl. Acad. Sci.* 95:1552-1555.
- Hwang, J. S., K. M. Lai, and K. C. Hsu. 2007. Changes in textural and rheological properties of gels from tilapia muscle proteins induced by high pressure and setting. *Food Chem.* 104:746-753.
- Iwasaki, T., K. Noshiroya, N. Saitoh, K. Okano, and K. Yamamoto. 2006. Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. *Food Chem.* 95:474:483.
- Jaenicke, R. and G. Böhm. 1998. The stability of proteins in extreme environments. *Curr. Opin. Struct. Biol.* 8:738-748.
- Janson, J. and L. Rydén. 1998. Protein Purification: Principles, High-resolution Methods, and Applications. J. Janson and L. Rydén, eds. John Wiley & Sons, Inc., USA.
- Kahn, A. W. 1975. Effect of chemical treatments causing rapid onset of rigor on tenderness of poultry breast meat. *J. Agric. Food Chem.* 23:449-451.
- Kannan, G., J. L. Heath, C. J. Wabeck, S. L. Owens, and J. A. Mench. 1998. Elevated plasma corticosterone concentrations influence the onset of rigor mortis and meat color in broilers. *Poult. Sci.* 77:322-328.

- Kato, A., Y. Osako, N. Matsudomi, and K. Kobayashi. 1983. Changes in emulsifying and foaming properties of proteins during heat denaturation. *Agric. Biol. Chem.* 47:33-37.
- Kerry, J., J. Kerry, and D. Ledward. 2002. *Meat Processing: Improving Quality*. J. Kerry, J. Kerry, and D. Ledward, eds. Woodhead Publishing Limited and CRC Press, Boca Raton, FL, USA.
- Kinsella, J. E. and D. M. Whitehead. 1989. Proteins in whey: Chemical, physical, and functional properties. *Adv. Food. Nutr. Res.* 33:343-438.
- Kinsman, D. M., A. W. Kotula, and B. C. Breidenstein. 1994. Pages 51-53 in *Muscle Foods: Meat Technology and Seafood Technology*. D. M. Kinsman, A. W. Kotula, and B. C. Breidenstein, eds. Chapman & Hall Inc., Great Britain.
- Knorr, D. 1993. Effects of high-hydrostatic-pressure processes on food safety and quality. *Food Technol.* 47:156-161.
- Ko, W. C. 1996. Effect of high pressure on gelation of meat paste and inactivation of actomyosin Ca-ATPase prepared from milkfish. *Fish Sci.* 62:101-104.
- Ko, W. C., C. L. Jao, and K. C. Hsu. 2003. Effect of hydrostatic pressure on molecular conformation of Tilapia (*Oreochromis niloticus*) myosin. *J. Food Sci.* 68:1192-1195.
- Ko, W., C. Yu, and K. Hsu. 2007. Changes in conformation and sulfhydryl groups of tilapia actomyosin by thermal treatment. *LWT.* 40:1316-1320.
- Koohmaraie, M., J. E. Schollmeyer, and T. R. Dutson. 1986. Effect of low-calcium-requiring calcium activated factor on myofibrils under varying pH and temperature conditions. *J. Food Sci.* 51:28-32.

- Kuchel, P. W., S. B. Easterbrook-Smith, V. Gysbers, D. P. Hancock, J. M. Guss. 2009. Proteins. Pages 95-134 in Schaum's Outline of Biochemistry. 3rd ed. P. W. Kuchel, S. B. Easterbrook-Smith, V. Gysbers, D. P. Hancock, J. M. Guss, eds. McGraw-Hill Companies, Inc, USA.
- Küchenmeister, U., G. Kuhn, J. Wegner, G. Nürnberg, and K. Ender. 1999. Postmortem changes in Ca²⁺ transporting proteins of sarcoplasmic reticulum in dependence on malignant hyperthermia status in pigs. *Mol. Cell. Biochem.* 195:37-46.
- Lawrie, R. A. and D. A. Ledward. 2006. Chemical and biochemical constitution of muscle. Pages 75-127 in Lawrie's Meat Science. 7th ed. R. A. Lawrie and D. A. Ledward, eds. Woodhead Publishing Limited and CRC Press LLC, Boca Raton, FL, USA.
- LeBlanc, E. L. and R. J. LeBlanc. 1992. Determination of hydrophobicity and reactive groups in proteins of cod (*Gadus morhua*) muscle during frozen storage. *Food Chem.* 43:3-11.
- Lin, H., H. C. Jiao., J. Buyse., and E. Decuyper. 2006. Strategies for preventing heat stress in poultry. *W. Poult. Sci. J.* 62:71-86.
- Lundström, K., A. Andersson, and I. Hansson. 1996. Effect of the RN gene on technological and sensory meat quality in crossbred pigs with Hampshire as terminal sire. *Meat Sci.* 42:145-153.
- Lyon, C. E. and R. J. Buhr. 1999. Biochemical basis of meat texture. Pages 99-126 in Poultry Meat Science. R. I. Richardson and G. C. Mead, eds. CAB International, NY, USA.

- Macfarlane, J. J. 1974. Pressure-induced solubilisation of meat proteins in saline solution. *J. Food Sci.* 39:542-547.
- Macfarlane, J. J. and I. J. McKenzie. 1976. Pressure-induced solubilization of myofibrillar proteins. *J. Food Sci.* 41:1442-1446.
- Mallia, J. G., S. Barbut, J.-P. Vaillancourt, S. W. Martin, and S. A. McEwen. 2000. A dark, firm dry-like condition in turkeys condemned for cyanosis. *Poult. Sci.* 79:281-285.
- McCurdy, R. D., S. Barbut, and M. Quinton. 1996. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. *Food Res. Int.* 29:363-366.
- McKee, S. R. and A. R. Sams. 1997. The effect of seasonal heat stress on rigor development and the incidence of pale, exudative turkey meat. *Poult. Sci.* 76:1616-1620.
- McKee, S. R. and A. R. Sams. 1998. Rigor mortis development at elevated temperatures induces pale exudative turkey meat characteristics. *Poult. Sci.* 77:169-174.
- Messens, W., J. Van Camp, and A. Huyghebaert. 1997. The use of high pressure to modify the functionality of food proteins. *Trends Food Sci. Technol.* 8:107-112.
- Messens, W. and J. Van Camp. 2003. High-pressure processing to improve dairy product quality. Pages 310-332 in *Diary Processing: Improving Quality*. G. Smit, ed. Woodhead Publishing Limited and CRC Press, Boca Raton, FL, USA.

- Mickelson, J. R., E. M. Gallant, W. E. Rempel, K. M. Johnson, L. A. Litterer, B. A. Jacobson, and C. F. Louis. 1989. Effects of the halothane-sensitivity gene on sarcoplasmic reticulum function. *Am. J. Physiol.* 25:C781-C794.
- Monin, G., P. Sellier, L. Ollivier, R. Goutefongea, and J. P. Girard. 1981. Carcass characteristics and meat quality of halothane negative and halothane positive Pietrain pigs. *Meat Sci.* 5:413-423.
- Monin, G., A. Mejenes-Quijano, A. Talmant, and P. Sellier. 1987. Influence of breed and muscle metabolic type on muscle glycolytic potential and meat pH in pigs. *Meat Sci.* 20:149-158.
- Morild, E. 1981. The theory of pressure effects on enzymes. *Adv. Protein Chem.* 34:93-166.
- Offer, G. and P. Knight. 1988. The structural basis of water-holding in meat. Pages 173-243 in *Developments in Meat Science*. R. A. Lawrie, ed. Elsevier Applied Science, NY, USA.
- Offer, G. 1991. Modelling of the formation of pale, soft and exudative meat: Effects of chilling regime and rate and extent of glycolysis. *Meat Sci.* 30:157-184.
- Okamoto, M., Y. Kawamura, and R. Hayashi. 1990. Application of high pressure to food processing: Textural comparison of pressure- and heat-induced gels of food proteins. *Agric. Biol. Chem.* 54:183-189.
- Ottini, L., G. Marziali, A. Conti, A. Charlesworth, and V. Sorrentino. 1996. α and β isoforms of ryanodine receptor from chicken skeletal muscle are homologues of mammalian RyR1 and RyR3. *Biochem. J.* 315:207-216.

- Ouali, A., N. Garrel, A. Obled, C. Deval, and C. Valin. 1987. Comparative action of cathepsins D, B, H, L and of a new lysosomal cysteine proteinase on rabbit myofibrils. *Meat Sci.* 19:83-100.
- Owens, C. M. and A. R. Sams. 2000. The influence of transportation on turkey meat quality. *Poult. Sci.* 79:1204-1207.
- Owens, C. M., E. M. Hirschler, S. R. McKee, R. Martinez-Dawson, and A. R. Sams. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. *Poult. Sci.* 79:553-558.
- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Pauling, L. 1960. Chemical bond. Page 85 in *The Nature of the Chemical Bond*. 3rd ed. L. Pauling, ed. Cornell University Press, NY, USA.
- Pelegrine, D. H. G. and C. A. Gasparetto. 2005. Whey proteins solubility as function of temperature and pH. *Lebensm.-Wiss. U.-Technol.* 38:77-80.
- Pérez-Mateos, M. and P. Montero. 1997. High-pressure-induced gel of sardine (*Sardina pilchardus*) washed mince as affected by pressure-time-temperature. *J. Food Sci.* 62:1183-1188.
- Petracci, M., M. Betti, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. *Poult. Sci.* 83:2086-2092.
- Poultry Marketplace. 2010. World future outlook of the chicken meat market. http://www.agr.gc.ca/poultry/prindc2_eng.htm.

- Pratt, C. W. and K. Cornely. 2004. Myoglobin and hemoglobin: A study of protein structure and function. Pages 90-135 in *Essential Biochemistry*. C. W. Pratt and K. Cornely, eds. John Wiley & Sons, Inc., USA.
- Ptitsyn, O. B. and V. N. Uversky. 1994. The molten globule is a third thermodynamical state of protein molecules. *FEBS Lett.* 341:15-18.
- Puolanne, E. J., M. H. Ruusunen, and J. I. Vainionpää. 2001. Combined effects of NaCl and raw meat pH on water-holding in cooked sausage with and without added phosphate. *Meat Sci.* 58:1-7.
- Puri, D. 2006. Amino acids, peptides, and proteins. Pages 61-95 in *Textbook of Biochemistry*. D. Puri, ed. Elsevier, New Delhi, India.
- Ramakrishnan, S., K. G. Prasanna, and R. Rajan. 2001. Composition and structure of muscle and biochemistry of muscular contraction. Pages 177-182 in *Textbook of Medical Biochemistry*. 3rd ed. S. Ramakrishnan, K. G. Prasanna, and R. Rajan, eds. Orient Longman Private Ltd., India.
- Rasmusen, B. A. and L. L. Christian. 1976. H blood types in pigs as predictors of stress susceptibility. *Science.* 191:947-948.
- Rathgeber, B. M., J. A. Boles, and P. J. Shand. 1999. Rapid postmortem pH decline and delayed chilling reduce quality of turkey breast meat. *Poult. Sci.* 78:477-484.
- Rust, R. E. 1987. *Science of Meat and Meat Products*. 3rd ed. J. F. Schweigert and B. S. Schweigert, eds. Food and Nutrition Press Inc., Trumbull, CT.

- Santos, C., L. C. Roserio, H. Goncalves, and R. S. Melo. 1994. Incidence of different pork quality categories in a Portuguese slaughterhouse: A survey. *Meat Sci.* 38:279-287.
- Shimada, K. and J. C. Cheftel. 1989. Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. *J. Agric. Food Chem.* 37:161-168.
- Sikes, A. L., A. B. Tobin, and R. K. Tume. 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Inn. Food Sci. Emerg. Technol.* 10:405-412.
- Silva, J. L. 1993. Pressure stability of proteins. *Annu. Rev. Phys. Chem.* 44:89-113.
- Smith, D. P. and D. L. Fletcher. 1992. Post-mortem biochemistry of Pekin ducklings and broiler chicken pectoralis muscle. *Poult. Sci.* 71:1768-1772.
- Smith, D. M. 2001. Functional properties of muscle proteins in processed poultry products. Pages 181-194 in *Poultry Meat Processing*. A. R. Sams, ed. CRC Press, Taylor & Francis, Boca Raton, FL, USA.
- Strasburg, G. M. and W. Chiang. 2009. Pale, soft, exudative turkey - The role of ryanodine receptor variation in meat quality. *Poult. Sci.* 88:1497-1505.
- Suzuki, A., T. Tsuchiya, S. Ohwada, and H. Tamate. 1985. Distribution of myofiber types in thigh muscles of chickens. *J. Morph.* 185:145-154.
- Swatland, H. J. 1990. A note on the growth of connective tissues binding turkey muscle fibers together. *Can. Inst. Food Sci. Technol. J.* 23:239-241.

- Tanaka, N., C. Ikeda, K. Kanaori, K. Hiraga, T. Konno, and S. Kunugi. 2000. Pressure effect on the conformational fluctuation of apomyoglobin in the native state. *Biochem.* 39:12063-12068.
- Tarté, R. and C. M. Amundson. 2006. Protein interactions in muscle foods. Pages 196-282 in *Ingredient Interactions: Effect on Food Quality*. 2nd ed. A. G. Gaonkar and A. McPherson, eds. Taylor & Francis Group, LLC, Boca Raton, FL, USA.
- Tauscher, B. 1995. Pasteurization of food by hydrostatic high pressure: chemical aspects. *Z. Leb. Unt. For.* 200:3-13.
- Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie, and D. E. Goll. 1995. Is Z-disk degradation responsible for postmortem tenderization? *J. Anim. Sci.* 73:1351-1367.
- Tewari, G. 2007. High-pressure processing. Pages 203-240 in *Advances in Thermal and Non-thermal Food Preservation*. G. Tewari and V. K. Juneja, eds. Blackwell Publishing, USA.
- Toldrá, F. 2002. Description of main muscle characteristics. Pages 7-26 in *Dry-cured Meat Products*. F. Toldrá, ed. Food & Nutrition Press, Inc., USA.
- Totosaus, A., J. G. Montejano, J. A. Salazar, and I. Guerrero. 2002. A review of physical and chemical protein-gel inductions. *Int. J. Food Sci. Technol.* 37:589-601.
- Updike, M. S., H. N. Zerby, J. C. Sawdy, M. S. Lilburn, G. Kaletunc, and M. P. Wick. 2005. Turkey breast meat functionality differences among turkeys selected for body weight and/or breast yield. *Meat Sci.* 71:706-712.

- Urich, K. 1994. Proteins of muscle and the cytoskeleton. Pages 331-375 in Comparative Animal Biochemistry. K. Urich, ed. Springer-Verlag, Berlin, Heidelberg.
- Valin, C., H. R. Cross, and S. B. Smith. 1992. Tissue development in relation to meat quality. Pages 131-150 in Beef Cattle Production. R. Jarrige and C. Béranger, eds. Elsevier Science Publishers, Netherlands.
- Van Laack, R. L. and R. G. Kauffman. 1999. Glycolytic potential of red, soft, exudative pork longissimus muscle. *J. Anim. Sci.* 77:2971-2973.
- Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* 79:1057-1061.
- Van Loey, A. and M. Hendrickx. 2002. High pressure processing. Pages 433-461 in The Nutrition Handbook for Food Processors. C. J. K. Henry and C. Chapman, eds., Woodhead Publishing Ltd., Boca Raton, FL, USA.
- Velleman, S. G. 2002. Role of the extracellular matrix in muscle growth and development. *J. Anim. Sci.* 80:E8-E13.
- Venugopal, V. 2006. Seafood Processing, Adding Value through Quick Freezing, Retortable Packaging and Cook-Chilling. V. Venugopal, ed. CRC Press, Taylor & Francis, Boca Raton, FL, USA.
- Vojdani, F. 1996. Solubility. Pages 11- 60 in Methods of Testing Protein Functionality. G. M. Hall, ed. Blackie Academic & Professional, London, UK.
- Wang, B. 2006. Handbook of Food Science, Technology, and Engineering. Y. H. Hui, ed. CRC Press, Taylor and Francis, Boca Raton, FL, USA.

- Wang, R. R., X. J. Pan, and Z. Q. Peng. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of *pectoralis majors* in broilers. *Poult. Sci.* 88:1078-1084.
- Warriss, P. D., E. A. Bevis, and P. J. Ekins. 1989. The relationships between glycogen stores and muscle ultimate pH in commercially-slaughtered pigs. *Br. Vet. J.* 145:378-383.
- Warriss, P. D. 1994. Pig handling: Guidelines for the handling of pigs antemortem. *Meat Focus Int.* 4:491-494.
- Warriss, P. D. 2003. Optimal lairage times and conditions for slaughter pigs: A review. *Vet. Rec.* 153:170-176.
- Whitaker, J. R. and S. R. Tannenbaum. 1977. Food proteins. Pages 121-174, 245-259 in *Food Proteins*. J. R. Whitaker and S. R. Tannenbaum, eds. Avi Pub. Co, USA.
- Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poult. Sci.* 81:579-584.
- Xia, X., B. Kong, Q. Liu, and J. Liu. 2009. Physicochemical change and protein oxidation in porcine *longissimus dorsi* as influenced by different freeze-thaw cycles. *Meat Sci.* 83:239-245.
- Xiong, Y. L. 1997. Structure-function relationships of muscle proteins. Pages 341-392 in *Food Proteins and Their Applications*. S. Damodaran and A. Paraf, eds. Marcel Dekker Inc., NY, USA.

- Xiong, Y. L. 2004. Muscle proteins. Pages 100-122 in *Proteins in Food Processing*. R. Y. Yada, ed. CRC Press, Taylor and Francis, Boca Raton, FL, USA.
- Yahav, S., S. Goldfield, I. Plavnik, and S. Hurwitz. 1995. Physiological responses of chickens and turkeys to relative humidity during exposure to high ambient temperature. *J. Therm. Biol.* 20:245-253.
- Yaldagard, M., S. A. Mortazavi, and F. Tabatabaie. 2008. The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects. *Afr. J. Biotech.* 7:2739-2767.
- Zayas, J. F. 1997. *Functionality of proteins in food*. J. F. Zayas, ed. Springer-Verlag, Berlin Heidelberg.
- Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.
- Ziober, I. L., F. G. Paião, D. F. Marchi, L. L. Coutinho, E. Binneck, A. L. Nepomuceno, and M. Shimokomaki. 2010. Heat and chemical stress modulate the expression of the α -RZR gene in broiler chickens. *Genet. Mol. Res.* 9:1258-1266.

CHAPTER 3. EFFECT OF ULTIMATE pH AND FREEZING ON THE BIOCHEMICAL PROPERTIES OF PROTEINS IN TURKEY BREAST MEAT¹

3.1 INTRODUCTION

The turkey industry is facing a major challenge due to increasing occurrence of pale, soft, exudative (PSE)-like meat similar to the PSE condition observed in the swine industry (Owens et al., 2009). In swine, a genetic mutation on the ryanodine receptor, a protein involved in regulation of calcium metabolism in muscle, plays a major role in the development of PSE meat which is related to stress susceptibility (de Vries et al., 2000). Strasburg and Chiang (2009) also emphasized the role of the ryanodine receptor in the development of a PSE-like condition in turkeys. However, to date, such a mutation as it was observed in pigs has not been identified in poultry species. It is generally believed that turkeys and broiler chickens behave differently from pigs in regard to PSE expression due to genetic differences between mammalian and avian species (Smith and Northcutt, 2009). There are also other factors which influence the development of PSE-like turkey meat, such as season, environment, heat stress, feed quality, and pre- and post-slaughter handling practices (Betti et al., 2009; Owens et al., 2009; Zhang et al., 2011).

In the turkey industry, resolving the PSE problem has only been a focus in the last decade, while it was studied in the pork industry for the last three decades (Barbut et al., 2005). It has been reported that the commercial incidence of PSE-

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like turkey meat ranges from approximately 5 to 40% of the birds in slaughterhouses (Barbut, 1996; Owens et al., 2000; Owens et al., 2009). Owens et al. (2009) suggested that the PSE problem costs the turkey industry at least U.S. \$200 million per year due to reduced protein functionality in further processed products. Conventional intervention methods used by industry to reduce the occurrence of PSE-like poultry meat are mainly aimed at stress reduction (Barbut et al., 2005).

In the past, several authors (Barbut, 1996; Owens et al., 2000; Fraqueza et al., 2006) have identified and classified PSE-like poultry meat, particularly in turkey by using meat selection and classification criteria, based on color and pH (Owens et al., 2009). In previous studies, Qiao et al. (2001) and Bianchi et al. (2005) have sorted broiler breasts into different colors (pale, normal and dark) using color classification by measuring lightness (L^*) values in commercial processing plants. They found that there was color variation among region, season, diet and flock. Fraqueza et al. (2006) have also conducted a study in a Portuguese slaughterhouse on a large population of turkey and reported the relationship between ultimate pH at 24 h *post mortem* (pH_{24}) and L^* values and found only 9% of variability associated with these two factors. Therefore, color alone is not a useful indicator of PSE-like poultry meat (Smith and Northcutt, 2009). Fraqueza et al. (2006) also observed poor correlation between pH at 15 minutes *post mortem* (pH_{15}) and functional properties such as drip loss and cooking loss. Hence, pH_{24} was considered an important criteria for classifying PSE-like meat (Fraqueza et al., 2006). Different pH_{24} has a profound influence on the functional properties of

poultry meat. Low pH_{24} results in meat with poor water holding capacity (WHC) and poorer texture (Owens et al., 2000). On the other hand, high pH_{24} results in high pH meat with a shorter shelf life (Allen et al., 1997). The major problem with high pH meat is its darker appearance which affects the color of processed products and consumer acceptability. Other factors, such as freezing can also cause biochemical and quality changes in meat.

Research studies to determine important biochemical and functional properties of proteins in turkey breast meat with different pH_{24} are scarce. Since the PSE problem is a growing concern in the turkey industry, it is important to further understand the relationships between these properties. Yet, details on the characteristics and occurrences of dark, firm, dry (DFD)-like turkey meat are not available. As the demand for further processed products increases, there is a need for industry to search for ways to utilize these types of meats depending on their protein functionality. Hence, our aim was to conduct a study to determine the biochemical and functional properties of proteins in turkey breast meat with different pH_{24} in fresh and frozen storage.

3.2 MATERIALS AND METHODS

3.2.1 Materials and sample preparation

Sixteen skinless, boneless breast fillets from Hybrid Tom turkeys were collected from a local processing plant (Lilydale Inc., Edmonton, Alberta, Canada), at 24 h *post-mortem* for each class (pale, normal, and dark), with a total number of 48, using color measurement based on lightness (L^*) values as reported by Zhang and Barbut (2005). The average turkey age was 116 days and the

average flock weight was 14.3 kg. The samples were labeled and placed individually in plastic bags, packed on ice and transported to the laboratory. L^* and pH_{24} were measured again on all 48 breast samples in the laboratory. Within each class (pale, normal, and dark), 8 breast samples were further selected according to their ultimate pH. In this experiment, because ultimate pH has been indicated as a valid tool to differentiate protein functionality, extremely low and extremely high pH meat was selected. In summary, the color and pH characteristics are reported in Table 3.1 and a photograph of the breast samples is shown in Figure 3.1. Samples were within the following range: pale ($L^* > 51$, $pH < 5.8$), normal ($45 < L^* < 49$, $5.9 < pH < 6.0$), and dark ($L^* < 46$, $pH > 6.2$), and referred to as low, normal, and high pH meat, respectively. Water holding capacity measured as expressible moisture (EM) was also determined on the three classes of meat and is reported in Table 3.1. Each breast was labeled according to the class and was minced individually in a Kitchen-Aid food processor (Model KFP 7500B, KitchenAid, St. Joseph, MI, USA) for 2 min. Each minced sample was equally split into two portions and was labeled for fresh and frozen analyses. The food processor was pre-chilled and operated in a cold room (4°C) to prevent the temperature of the samples from exceeding 10°C throughout the mixing process. All the analyses were carried out both in fresh and frozen samples. Minced meat was stored at 4°C (for four days) until the analyses in fresh condition was carried out. Frozen storage analyses were carried out on minced meat that was vacuum packaged in polyethylene bags and stored at -30°C for 3 weeks and thawed overnight at 4°C .

Table 3.1. Physical properties of low, normal, and high pH meat¹

Measurement	Classes of Meat		
	Low pH meat	Normal pH meat	High pH meat
L* (24 h)	53.3 ± 1.4 ^a	47.7 ± 1.3 ^b	44.2 ± 1.6 ^c
a* (24 h)	3.85 ± 1.31 ^a	4.80 ± 0.72 ^a	4.70 ± 0.50 ^a
b* (24 h)	2.64 ± 1.01 ^a	1.55 ± 1.27 ^{a,b}	1.13 ± 0.43 ^b
pH ₂₄	5.70 ± 0.04 ^c	6.00 ± 0.06 ^b	6.32 ± 0.15 ^a
Expressible moisture (%)	23.5 ± 5.9 ^a	18.2 ± 1.7 ^b	13.9 ± 1.7 ^b

¹Results are presented as means ± standard deviations (n=8).

Dissimilar superscripts in the same column denote significant difference ($P < 0.05$). L* = lightness; a* = redness; b* = yellowness.

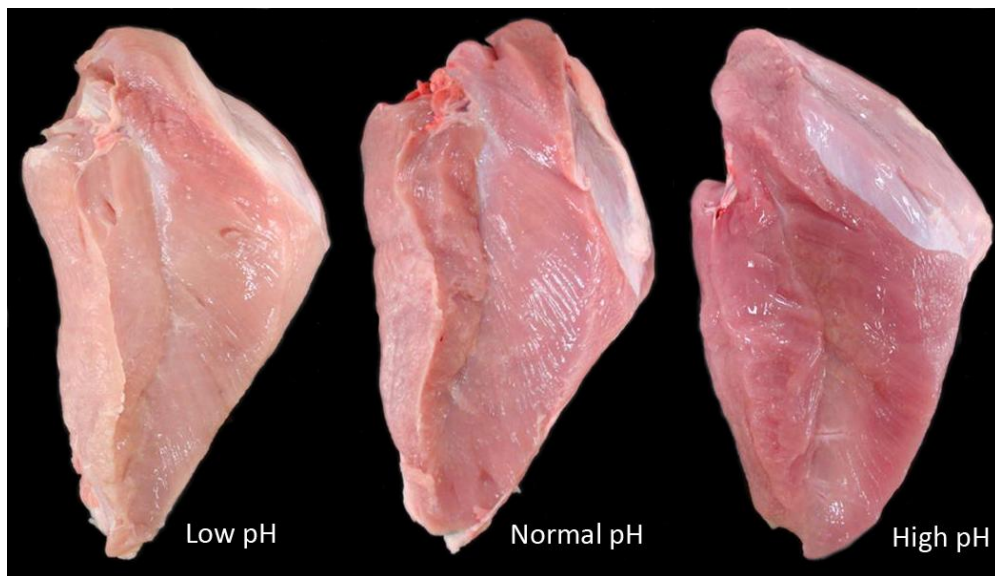


Figure 3.1. Photograph of low, normal, and high pH turkey breast meat at 24 h *post mortem*.

3.2.2 Color measurement

The colorimeter, Minolta CR-400 (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) was calibrated using a standard white ceramic tile. Color was measured on the internal side of turkey breasts in an area free of obvious color defects so as to get a uniform color reading with illuminant D65 as the light source. L*, a* and b* refer to lightness, redness, and yellowness, respectively.

3.2.3 pH measurement

Approximately 5 g of minced turkey breast meat was homogenized with 45 mL of distilled water and the pH of the homogenate was determined using a pH meter (UB-10, Ultra Basic pH meter, Denver Instrument, Bohemia, NY, USA).

3.2.4 Water holding capacity

The water holding capacity of the samples as measured by expressible moisture was determined according to the method as described by Omana et al. (2010). Raw meat (300 mg) was placed on a pre-weighed Whatman No. 1 filter paper and was placed between two glass plates. Using the texture profile analyzer (TA-XT Express, Stable micro systems, Ltd., Surrey, England), in adhesive test mode, the samples were tested with a target force of 1000 g for 2 min, which was sufficient to express the water content. After the test, the filter paper with the absorbed water was immediately weighed. Expressible moisture was measured as the quantity of water released per gram of meat and was expressed as a percentage.

$$\text{Expressible moisture (\%)} = \frac{(\text{Weight of wet paper} - \text{Weight of dry paper})}{\text{Weight of meat}} \times 100$$

3.2.5 Calcium activated adenosine triphosphatase (Ca²⁺-ATPase) enzyme activity

Ca²⁺-activated ATPase enzyme activity was determined according to the method described by Noguchi and Matsumoto (1970) with modifications by Dileep et al. (2005). Ca²⁺-activated ATPase enzyme activity was determined and expressed as µg of inorganic phosphorous (Pi)/mg protein/min at 27°C. One gram of meat was ground with 10 mL of 50 mM glycine-NaOH buffer (pH 9.2), and the slurry was filtered through Whatman No.1 filter paper. This filtrate was used as enzyme solution. The reaction mixture was made by mixing 0.06 mL of ATP solution (0.05 M), 0.4 mL CaCl₂ (0.1 M), 2 mL buffer (0.05 M glycine-NaOH, pH 9.2) and 0.4 mL of meat extract and was made up to 4.0 mL with buffer. After incubating at 27°C for 5 min, 2 mL of 15% trichloroacetic acid (TCA) was added to stop the reaction. The blank was carried out by addition of 15% TCA before the addition of enzyme. The mixture was filtered through Whatman No.1 filter paper and the inorganic phosphorous released was estimated according to the procedure described by Tausky and Shorr (1952).

3.2.6 Protein solubility

Sarcoplasmic and total protein solubility was determined according to the method as described by Van Laack et al. (2000) with slight modifications. For sarcoplasmic protein solubility, 2 g of meat was homogenized with 20 mL 0.03 M phosphate buffer (pH 7.4). After overnight storage at 4°C, the homogenate was filtered through Whatman No.1 filter paper. The protein concentration of the filtrate was assessed using the Biuret procedure (Gornall et al., 1949) with bovine

serum albumin (BSA) as standard. For total protein solubility, 1 g of meat was homogenized with 20 mL 0.05 M phosphate buffer containing 0.55 M KI (pH 7.4). Homogenates were stored, filtered, and analyzed for protein concentration as described for the sarcoplasmic protein solubility.

3.2.7 Protein surface hydrophobicity

Surface hydrophobicity of sarcoplasmic and myofibrillar proteins was determined according to the method as described by Kim et al. (2003) using hydrophobic fluorescent probes, 1-anilino-8-naphthalene-sulfonate (ANS; 8mM in 0.1M phosphate buffer, pH 7.4). The ANS probe is composed of aromatic rings and is useful for determining aromatic hydrophobicity (Hayakawa and Nakai, 1985). Sarcoplasmic protein solution was prepared by homogenizing 2 g of meat with 40 mL of 0.03 M phosphate buffer (pH 7.4). Homogenates were centrifuged using Avanti J-E refrigerated centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) at 15,300 x g for 15 min at 4°C and filtered through Whatman No. 1 filter paper. The supernatant was used as protein solution and serially diluted with the same buffer to a final volume of 4 mL to obtain a protein concentration ranging from 0.008 to 0.03%. The protein concentration was determined by the Biuret procedure (Gornall et al., 1949). ANS solution (20 µl) was added to the sample solution and the relative fluorescence intensity (RFI) of ANS protein was measured with a spectrofluorometer (Thermo Electron Fluoroskan Ascent, Vantaa, Finland) using 355 nm and 460 nm as the excitation and emission wavelengths, respectively. The net RFI was obtained by subtracting the RFI of each sample measured without ANS from that with ANS. The initial slope (H_0) of the RFI

against protein concentration (expressed in %), calculated by linear regression analysis, was used as an index of protein surface hydrophobicity. Myofibrillar protein solution was prepared after separation of sarcoplasmic proteins. The sediment was further homogenized with 40 mL of 0.05 M phosphate buffer containing 0.55 M KI (pH 7.4). Homogenates were centrifuged at 15,300 x g for 15 minutes at 4°C and filtered through Whatman No.1 filter paper. The rest of the assay was similar to that of sarcoplasmic protein surface hydrophobicity.

3.2.8 Reactive (free) and total sulfhydryl content

Reactive (R-SH) sulfhydryl content was determined according to the method as described by Kim et al. (2003). 25 mL of tris-glycine buffer (pH 8.0) containing 5 mM EDTA was added to 2.5 g of meat and was intermittently vortexed for 20 min to obtain a homogenized mixture. The homogenate was filtered and to 1 mL of filtrate, 4 mL of tris-glycine buffer and an aliquot (50 µl) of Ellman's reagent (10 mM 5,5'-dithiobis (2-nitrobenzoic acid)) was added to determine the surface sulfhydryl content. The protein content of the filtrate was determined using the Biuret procedure (Gornall et al., 1949). The mixture was kept in a cold room (4°C) for 1 hour with occasional stirring. Total (T-SH) sulfhydryl content was determined according to the method as described by Choi and Park (2002) with modifications. To 1 mL of filtrate, 4 mL of 10 M urea and 50 µl of Ellman's reagent were added. The mixture was kept at room temperature (25°C) for 1 hour with occasional stirring. The absorbance was measured at 412 nm using a spectrophotometer (V-530, Jasco Corporation, Tokyo, Japan). The

sulfhydryl contents were determined using the extinction coefficient of $13,600 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as $\mu\text{mol/g}$ of protein.

3.2.9 Protein carbonyl content

Protein carbonyl content was determined according to the method as described by Oliver et al. (1987) as per modifications of Xia et al. (2009). Carbonyl groups were detected by reactivity with 2,4-dinitrophenylhydrazine (DNPH) to form protein hydrazones. Protein solution was prepared as per the method used for total protein solubility. The protein solution was diluted to a protein concentration ranging from 0.7 to 1.0 mg/mL and was precipitated with 10% TCA (w/v; final concentration). After centrifugation (2000 x g, 10 min, 4°C), samples (pellets) were treated with 0.2% DNPH (w/v) in 2 M HCl, while 2 M HCl was added to blanks. The solutions were further incubated at 25°C for 1 hour with occasional agitation. After precipitation with 10% TCA, the solutions were further centrifuged. The pellets were washed twice with 1 mL of ethanol-ethyl-acetate (1:1, v/v) and the solution was precipitated with 10% TCA and centrifuged. The sediment after centrifugation was then dissolved in 2 mL of 6 M guanidine in 20 mM sodium phosphate buffer (pH 6.5). Absorbance was measured at 365 nm for the DNPH-treated sample against HCl as control. Protein concentration was calculated from the absorbance at 280 nm in the HCl control using a standard bovine serum albumin in guanidine. The amount of carbonyl was expressed as nmol of DNPH fixed/mg of protein using an absorption coefficient of $22,000 \text{ M}^{-1} \text{ cm}^{-1}$ for protein hydrazones.

3.2.10 Statistical analysis

Each assay was carried out in 8 replications per meat type and was done at least in duplicate. Reported results represent an average of each experimental assay. The data were analyzed as a 3 x 2 factorial ANOVA using the Mixed procedure of SAS (SAS version 9.0, SAS Institute, Cary, NC, USA, 2006). The model tested the main effects for meat pH group (low, normal and high) and storage (fresh and frozen) as well as the interaction term using residual errors. Differences between group means were determined using HSD Tukey differences and were reported as significant at the $P < 0.05$ level. Pearson's correlation analysis was performed for relationship evaluation of data.

In order to further elucidate the relationship among pH, freezing and biochemical properties of proteins, a principal component analysis (PCA) was performed using the multivariate analysis option of Statistica 6.1 (StatSoft, Version 6.1, StatSoft. Inc., Tulsa, OK, USA, 2003). The matrix consisted of 48 objects (8 breast fillets per each pH x storage group) and 8 variables (Ca^{2+} -ATPase activity, sarcoplasmic and total protein solubility, reactive and total sulfhydryl content, sarcoplasmic and myofibrillar protein surface hydrophobicity and carbonyl content).

3.3 RESULTS AND DISCUSSION

3.3.1 Ca^{2+} -ATPase enzyme activity

ATPase activity is present in mammalian myosin and is regulated by the presence of calcium ions which indicate the integrity of myosin molecule (Binsi et al., 2007). The Ca^{2+} -ATPase activity of low, normal, and high pH meat as a

function of frozen storage has been studied. There was no significant ($P > 0.05$) difference in Ca^{2+} -ATPase activity among these three classes of meat in fresh condition (Table 3.2) indicating that the myosin globular head is not affected by pH_{24} . These results are quite surprising and seem to concur with the findings of Van Laack and Lane (2000), who reported that chicken myosin is quite resistant to denaturation compared to porcine myosin. However, there was a significant ($P < 0.0001$) reduction in Ca^{2+} -ATPase activity in the frozen samples compared to fresh samples. This may be due to freeze denaturation of the myosin molecule resulting in it losing its integrity. The decreased activity of the enzyme after freezing was due to the changes in the integrity of myofibrillar proteins (Binsi et al., 2007). Decrease in Ca^{2+} -ATPase activity during ice storage of Ribbonfish meat was reported to be due to change in conformation of enzyme or aggregation of myosin molecules (Dileep et al., 2005). Benjakul and Bauer (2000) also reported that the decrease in Ca^{2+} -ATPase activity may be associated with the oxidation of sulfhydryl groups on the myosin globular head.

A significant interaction between pH and freezing effects was also observed for Ca^{2+} -ATPase activity ($P < 0.05$) (Table 3.2). In the case of normal pH meat, the values were found to decrease drastically from 0.109 to 0.055 $\mu\text{g Pi/mg total protein/minute}$ after frozen storage. However, no statistical difference in Ca^{2+} -ATPase activity among the three different classes of meat was observed after freezing.

Table 3.2. Biochemical properties of turkey breast meat with different pH₂₄ as a function of storage (fresh and frozen)¹

		Ca ²⁺ -ATPase Activity (µg Pi/mg protein/min)	Total Protein Solubility (mg/g)	Sarcoplasmic Protein Solubility (mg/g)	Carbonyl Content (nmol/mg protein)	Reactive Sulfhydryl Content (µmol/g protein)	Total Sulfhydryl Content (µmol/g protein)	Sarcoplasmic Protein Surface Hydrophobicity (Ho)	Myofibrillar Protein Surface Hydrophobicity (Ho)
Classes of Meat (CM) (n=16)									
Low pH		0.085 ± 0.022	161 ± 33 ^b	74 ± 14 ^b	1.88 ± 0.24	36 ± 3 ^b	71 ± 9	160 ± 42	634 ± 78 ^b
Normal pH		0.082 ± 0.035	162 ± 40 ^b	81 ± 7 ^b	1.82 ± 0.31	38 ± 3 ^{a,b}	74 ± 8	165 ± 33	615 ± 79 ^b
High pH		0.077 ± 0.019	193 ± 39 ^a	99 ± 9 ^a	1.88 ± 0.19	39 ± 2 ^a	75 ± 5	165 ± 32	698 ± 78 ^a
Storage (S) (n=24)									
	Fresh	0.098 ± 0.024 ^a	206 ± 21 ^a	80 ± 16 ^b	1.72 ± 0.25 ^b	39 ± 2 ^a	78 ± 3 ^a	190 ± 22 ^a	629 ± 45
	Frozen	0.064 ± 0.015 ^b	138 ± 18 ^b	90 ± 12 ^a	2.00 ± 0.15 ^a	36 ± 3 ^b	69 ± 8 ^b	136 ± 22 ^b	669 ± 106
Interaction (CM x S) (n=8)									
Low pH	Fresh	0.100 ± 0.021 ^{a,b}	192 ± 13 ^b	65 ± 12 ^d	1.80 ± 0.28 ^{ab}	38 ± 2 ^{a,b}	76 ± 5 ^{a,b}	196 ± 20 ^a	598 ± 32 ^b
	Frozen	0.070 ± 0.012 ^{b,c,d}	131 ± 8 ^d	84 ± 7 ^{b,c}	1.97 ± 0.18 ^a	34 ± 3 ^b	66 ± 9 ^c	123 ± 19 ^b	670 ± 95 ^{a,b}
Normal pH	Fresh	0.109 ± 0.028 ^a	198 ± 17 ^b	79 ± 9 ^c	1.59 ± 0.27 ^b	39 ± 1 ^a	79 ± 2 ^a	193 ± 21 ^a	631 ± 18 ^{a,b}
	Frozen	0.055 ± 0.011 ^d	126 ± 12 ^d	82 ± 5 ^c	2.05 ± 0.07 ^a	37 ± 4 ^{a,b}	69 ± 9 ^{b,c}	137 ± 9 ^b	599 ± 104 ^b
High pH	Fresh	0.085 ± 0.016 ^{a,b,c}	229 ± 11 ^a	95 ± 8 ^{a,b}	1.78 ± 0.17 ^{a,b}	41 ± 2 ^a	78 ± 3 ^a	182 ± 25 ^a	657 ± 54 ^{a,b}
	Frozen	0.068 ± 0.019 ^{c,d}	157 ± 14 ^c	103 ± 7 ^a	1.99 ± 0.17 ^a	37 ± 1 ^{a,b}	71 ± 3 ^{a,b,c}	147 ± 29 ^b	738 ± 79 ^a
Source of Variation					P - Value				
CM		0.4972	<0.0001	<0.0001	0.6056	0.0132	0.1929	0.7537	0.0082
S		<0.0001	<0.0001	0.0001	<0.0001	0.0004	<0.0001	<0.0001	0.0693
CM x S		0.0274	0.3437	0.0240	0.1116	0.3782	0.6780	0.0512	0.0803

¹Results are presented as means ± standard deviations.

Means within column within each analysis with no common superscript differ significantly ($P < 0.05$).

3.3.2 Protein solubility

Protein solubility is a good indicator of protein denaturation and is fundamentally related to its hydrophobicity/hydrophilicity balance (Van Laack et al., 2000; Omana et al., 2010). In the present study, total and sarcoplasmic protein solubility of high pH meat were 193 mg/g and 99 mg/g, respectively, and were significantly ($P < 0.0001$) higher than that of low and normal pH meat (Table 3.2). No significant difference was found between low and normal pH meat on total and sarcoplasmic protein solubility. Earlier work on broiler/turkey breast and pork showed lower protein solubility in PSE meat compared to normal meat (Pietrzak et al., 1997; Van Laack et al., 2000; Barbut et al., 2005). However, in the present study, it was found that normal pH meat had the same extent of protein denaturation as low pH meat as shown by protein solubility. As indicated in the previous section on Ca^{2+} -ATPase activity, it seems possible that turkey muscle proteins are highly resistant to *post mortem* acidification. For instance, in their study, Van Laack and Lane (2000) demonstrated that extracted myofibrillar proteins from chicken *pectoralis profundus* suspended in buffer solutions with pH values of 5.4 and 6.5, and incubated at 40°C for 10, 20, and 60 min were not affected by any of the treatments in terms of solubility. The authors concluded that myosin denaturation was not the main determinant of a PSE condition in broiler chickens.

Freezing caused significant ($P < 0.0001$) reduction (33%) in total protein solubility (Table 3.2). This reduction may be related to the instability of proteins due to aggregation behavior during the freezing process. A reduction in solubility

was also found in chicken actomyosin after frozen storage, which was mainly due to the association/dissociation of actomyosin leading to formation of aggregates (Cofrades et al., 1996). However, sarcoplasmic protein solubility was significantly ($P = 0.0001$) increased in the frozen samples (90 mg/g) compared to fresh samples (80 mg/g). Farouk et al. (2003) found an increase in sarcoplasmic protein solubility in beef after freezing. Frozen storage results in freeze denaturation of muscle proteins (Benjakul et al., 2003), which ultimately causes drip loss during thawing. Thus, the water exuding from the meat contains a large amount of sarcoplasmic protein (water soluble proteins); hence after frozen storage, it can be easily extracted and its solubility appeared high. Moreover, after frozen storage, sarcoplasmic proteins became more folded preventing exposure of hydrophobic groups on the protein surface as shown by the decrease in protein surface hydrophobicity (refer to section 3.3.4.). Thus, this allowed more interaction of proteins with the surrounding water. Owen (1996) mentioned that the fewer the number of surface hydrophobic patches, the greater the solubility.

Sarcoplasmic protein solubility was significantly ($P < 0.05$) affected by the interaction between classes of meat and storage (Table 3.2). This significant interaction is mainly due to lower protein solubility in fresh low pH meat. In each class of meat, sarcoplasmic protein solubility increased in frozen samples compared to fresh samples, with the greatest increase observed in low pH meat. This may be related to corresponding decrease in sarcoplasmic protein surface hydrophobicity after frozen storage in each class of meat, with the greatest decrease in low pH meat.

3.3.3 Water holding capacity

Water holding capacity is an important property which determines meat quality and is used to evaluate PSE meat (Woelfel et al., 2002). Expressible moisture, a good indicator of water holding capacity, is the percentage of total water in the meat that can be expressed by applied force. In this study, the expressible moisture of normal and high pH meat were 18.2 and 13.9%, respectively, (Table 3.1); however, there was no statistical difference between them. On the other hand, the expressible moisture of low pH meat was 23.5%, and was significantly higher ($P < 0.05$) than that of normal and high pH meat. This is consistent with previous work, which demonstrated that lower muscle pH is associated with lower WHC (Barbut, 1993; Fraqueza et al., 2006). However, in the present study, low and normal pH meat had the same extent of protein denaturation as shown by protein solubility (Table 3.2). This may indicate that the surface polarity and/or hydrophobicity ratio of the proteins was the same (Kinsella, 1982) for low and normal pH meat, resulting in the same amount of protein solubilised by the buffer solution used during the extraction. Hence, protein denaturation may not be the most important factor in determining the lower water retention ability of low pH meat (Van Laack and Lane, 2000); other factors may also be involved. Recent studies conducted in poultry and pigs have shown that an increase in phospholipase A₂ (PLA₂) activity on sarcolemma and sarcoplasmic reticulum (Chen et al., 2010) and an increase in protease enzyme activity on myofilaments (Wilhelm et al., 2010) in PSE meat are linked to the reduced ability of muscle to hold intracellular water. Proteoglycans, which are

present in the extracellular matrix surrounding the muscle cell, may also play a role in influencing the water holding capacity of PSE-like turkey meat (Velleman, 1998). Fast growing turkeys of today may not have a proportional increase in the water holding proteoglycans in relation to the muscle fibers' size, thus being predisposing to this syndrome (Velleman, 1998).

3.3.4 Protein surface hydrophobicity

The stability of native protein structures involves hydrophobic interactions. Protein surface hydrophobicity relates to the extent of distribution of hydrophobic residues on the protein surface (Nakai, 1996). Factors such as pH can cause conformational changes in protein structure thus affecting hydrophobicity. Protein unfolding causes more exposure of hydrophobic amino acid residues to the surface leading to higher hydrophobicity (Omana et al., 2010). Hydrophobicity also plays a key role in influencing the functional properties of proteins. Sarcoplasmic protein surface hydrophobicity showed only marginal changes in the three classes of meat (Table 3.2), suggesting that exposure of hydrophobic groups was similar as a function of pH₂₄. However, a significant ($P < 0.0001$) decrease in sarcoplasmic protein surface hydrophobicity was observed in frozen samples when compared to fresh samples, indicating that these proteins become more folded or aggregated during freezing, which led to decreased exposure of hydrophobic groups on the surface.

Myofibrillar protein surface hydrophobicity was significantly ($P < 0.05$) higher in high pH meat compared to low and normal pH meat (Table 3.2). This can be explained through physiological changes which happen *post mortem*. The

native state of meat muscle proteins is at physiological pH between 6.8 to 7.4 (Warriss, 2010). The pH_{24} mean value of high pH meat was 6.3 and was much closer to the physiological pH than low and normal pH meat (Table 3.1). During *post mortem* acidification of muscles, pH falls and proteins denature (Barbut 1993). Thus, the extent of acidification in high pH meat is much less than that of low pH meat. Myofibrillar proteins in low pH meat are near the isoelectric point (pI), which is usually at pH 5.3 (Offer and Knight, 1988). When myofibrillar proteins reach their pI, they denature and lose the ability to bind water because actin and myosin molecules pack more closely together (Warriss, 2010). Thus, the highly folded structure of protein in low pH meat has led to decreased exposure of hydrophobic residues on the surface. Conversely, the pH of high pH meat is further away from pI than low and normal pH. Hence, the protein structure in the protein extractions from high pH meat is more unfolded as confirmed by the increase in myofibrillar protein surface hydrophobicity. However, no statistical differences in myofibrillar protein surface hydrophobicity were found between low and normal pH meat, indicating a similar pattern of protein folding. This result concurs with those observed for Ca^{2+} -ATPase activity and protein solubility (Table 3.2).

Although no statistical difference at the probability level of 0.05 was observed for myofibrillar protein surface hydrophobicity as a function of frozen storage, results showed a tendency to be significantly different ($P = 0.0693$); hence, after frozen storage, myofibrillar protein surface hydrophobicity had the highest value. Several authors (Hayakawa and Nakai, 1985; Li-Chan et al., 1985)

have reported that both the freezing process and frozen storage can cause an increase in hydrophobicity of myofibrillar proteins. Careche et al. (1998) also found an increase in surface hydrophobicity of chicken natural actomyosin (NAM) during freezing and frozen storage. This increase may be due to mild denaturation of muscle proteins without aggregation, which caused protein unfolding during freezing (Li-Chan et al., 1985; Farouk et al., 2003).

3.3.5 Reactive (free) and total sulfhydryl content

Sulfhydryl groups are one of the most reactive functional group in proteins. The reactive sulfhydryl groups of low pH meat were significantly ($P < 0.05$) lower than high pH meat (Table 3.2). This may be due to the fact that proteins are more folded and sulfhydryl groups are less exposed since the pH was near its isoelectric point. This is further confirmed by the hydrophobicity data. However, the amount of total sulfhydryl groups was not statistically different ($P > 0.05$) in low, normal and high pH meat. A significant decrease in reactive ($P < 0.05$) and total ($P < 0.0001$) sulfhydryl groups in frozen samples was observed compared to fresh samples (Table 3.2). This may be due to denaturation and aggregation of muscle proteins as a result of oxidation of cysteine thiol groups or disulfide interchange reactions, leading to the formation of disulfide bonds either within polypeptides or between polypeptides (Xia et al., 2009). Oxidation of thiol groups is evident from protein oxidation studies as revealed from carbonyl content analysis in the frozen samples (refer to section 3.3.6.). Soyer et al. (2010) also reported a decrease in total sulfhydryl groups in chicken leg and breast meat during frozen storage. In fresh condition, there were more reactive sulfhydryl

groups exposed than in frozen condition because of greater amount of total sulfhydryl groups present.

3.3.6 Protein carbonyl content

Carbonyl content was measured to evaluate the degree of protein oxidation. Stadtman (1990) showed that carbonyl compounds, which are formed by the preferential attack of reactive oxygen species on the side chain of amino acid residues, causes proteins to have a loss of catalytic activity and increased susceptibility to proteolytic degradation. In this study, protein oxidation was found to be of the same extent ($P > 0.05$) as a function of pH₂₄ as shown by similar amounts of carbonyl content in low, normal and high pH meat (Table 3.2). However, this result is in contrast to that of Wang et al. (2009), in which they found that low pH breast meat in broilers induced by pre-slaughter heat exposure was having higher protein oxidation compared to meat with higher pH. This may also indicate that turkey muscles possess a better protective system against oxidative stress than broiler chickens. Carbonyl content increased significantly ($P < 0.0001$) from 1.72 to 2.00 nmol/mg of protein after frozen storage, indicating that freezing induced more protein oxidation. These values were similar to those previously reported in turkey breast (Mercier et al., 1998). Freezing can rupture cellular organelles, thus releasing oxidative enzymes and pro-oxidants (Xia et al., 2009). Factors contributing to protein oxidation are processing and storage conditions, which may have a significant impact on texture and functional properties of muscle (Xia et al., 2009), leading to increase in water loss and

weaker protein gels (Xiong, 2000). The results clearly showed that freezing has a strong impact on protein oxidation in turkey breast meat.

3.3.7 Correlations

Pearson's correlation coefficients among Ca^{2+} -ATPase activity, sarcoplasmic and total protein solubility, sarcoplasmic and myofibrillar protein surface hydrophobicity, reactive and total sulfhydryl content, and carbonyl content are presented in Table 3.3. Ca^{2+} -ATPase activity was positively correlated ($r = 0.59$; $P < 0.0001$) with total protein solubility; which indicates that higher protein solubility tend to be associated with higher Ca^{2+} -ATPase activity. There was a significant ($r = -0.48$; $P < 0.01$) negative correlation of carbonyl content and total protein solubility. Rowe et al. (2004) observed the formation of carbonyl groups associated with an increase in protein aggregation and loss of protein solubility. Generally, protein solubility tends to decrease as hydrophobicity increases. However, hydrophobicity is not the sole factor which determines the functional behavior of a protein (Omana et al., 2010). This was further supported by a significant ($r = 0.70$; $P < 0.0001$) positive correlation of total protein solubility and sarcoplasmic protein protein hydrophobicity.

3.3.8 Multivariate analysis: Principal component analysis (PCA)²

The classical analyses of variance determine the cause and the effect of one dependent variable at a time while principal component analysis (PCA) is used to analyze joint effects of variables. For instance, when a number of dependent variables are measured in the experimental unit, PCA can be an

² *Principal component analysis (PCA) was carried out by Dr. M. Betti to facilitate discussion of results.*

Table 3.3. Pearson's correlation coefficients among Ca²⁺-ATPase activity, total and sarcoplasmic protein solubility, carbonyl content, reactive and total sulfhydryl content, and sarcoplasmic and myofibrillar protein surface hydrophobicity¹

Biochemical Parameters (n=48)	Ca ²⁺ -ATPase Activity	Total Protein Solubility	Sarcoplasmic Protein Solubility	Carbonyl Content	Reactive Sulfhydryl Content	Total Sulfhydryl Content	Sarcoplasmic Protein Surface Hydrophobicity	Myofibrillar Protein Surface Hydrophobicity
Ca²⁺-ATPase Activity								
Pearson's correlation	---	0.59***	-0.29	-0.26	0.33*	0.45**	0.66***	0.06
Total Protein Solubility								
Pearson's correlation	0.59***	---	0.05	-0.48**	0.48**	0.54***	0.70***	-0.02
Sarcoplasmic Protein Solubility								
Pearson's correlation	-0.29	0.05	---	0.13	-0.07	-0.23	-0.29	0.39**
Carbonyl Content								
Pearson's correlation	-0.26	-0.48**	0.13	---	-0.09	-0.36*	-0.38*	0.15
Reactive Sulfhydryl Content								
Pearson's correlation	0.33*	0.48**	-0.07	-0.09	---	0.89***	0.54***	0.16
Total Sulfhydryl Content								
Pearson's correlation	0.45**	0.54***	-0.23	-0.36*	0.89***	---	0.63***	0.19
Sarcoplasmic Protein Surface Hydrophobicity								
Pearson's correlation	0.66***	0.70***	-0.29	-0.38*	0.54***	0.63***	---	0.01
Myofibrillar Protein Surface Hydrophobicity								
Pearson's correlation	0.06	-0.02	0.39**	0.15	0.16	0.19	0.01	---

* significant correlations at $P < 0.05$; ** significant correlations at $P < 0.01$; *** significant correlations at $P < 0.0001$

¹Units of the parameters: Ca²⁺-ATPase activity = $\mu\text{g Pi/mg protein/min}$; total and sarcoplasmic protein solubility = mg/g ; carbonyl content = nmol/mg protein ; reactive and total sulfhydryl content = $\mu\text{mol/g protein}$; sarcoplasmic and myofibrillar protein surface hydrophobicity = Ho.

effective tool to identify the most important dependent variables described usually by a few components. In summary, PCA transforms the original dependent variables into new axes or principal components (PC), which are orthogonal and independent of each other so that the data presented in each axis (component) are uncorrelated with all the other components (Goonewardene et al., 2004). Each PC represents a linear combination of the original variables with the coefficients being the correlation (loading) between PCs and the original variables. Normally, only a few PCs are sufficient to describe the total variation (Smith, 1991). The size of the variation for each component (vector) is indicated by the size of the latent root or eigen value (Karlsson, 1992). Furthermore, PCA can give a global representation of the data in a two dimensional plane defined by two components which can be very useful to group objects with similar characteristics.

The results for PCA analyses are shown in Table 3.4 and Table 3.5. Two principal component analyses were extracted from the data describing 45.47% (PC1) and 18.70% (PC2) of the variation, respectively. In other words, 64.17% of the total variance in the eight variables measured could be condensed into two new variables (PCs). The first PC had high loadings for Ca^{2+} -ATPase activity, total protein solubility, carbonyl content, reactive and total sulfhydryl content, and sarcoplasmic protein surface hydrophobicity, while the second PC had high loadings for sarcoplasmic protein solubility, carbonyl content, reactive sulfhydryl content, and myofibrillar protein surface hydrophobicity. In PC1, sarcoplasmic protein surface hydrophobicity, reactive and total sulfhydryl content, total protein

Table 3.4. Principal component eigen values for the principal component analysis¹

Principal Components	Eigen Value	Proportion of Total Variance (%)	Cumulative Variance Proportion (%)
PC1	3.64	45.47	45.47
PC2	1.50	18.70	64.17
PC3	1.01	12.61	76.78
PC4	0.74	9.23	86.01
PC5	0.62	7.72	93.74
PC6	0.27	3.41	97.15
PC7	0.17	2.12	99.27
PC8	0.06	0.73	100.00

¹PC = principal component.

Table 3.5. Coefficients of the loading (eigen vectors) for the first two principal components (PC)¹

Dependent variables	PC1 (%)	PC2 (%)
Ca ²⁺ -ATPase	0.75_a (23)	-0.15 (7)
Total protein solubility	0.81_a (25)	0.06 (3)
Sarcoplasmic protein solubility	-0.29 (9)	0.72_a (32)
Carbonyl content	-0.54_a (17)	0.29_a (13)
Reactive sulfhydryl content	0.74_a (23)	0.36_a (16)
Total sulfhydryl content	0.86_a (27)	0.23 (10)
Sarcoplasmic protein surface hydrophobicity	0.87_a (27)	-0.07 (3)
Myofibrillar protein surface hydrophobicity	0.03 (1)	0.82_a (36)
Total loadings (absolute)	3.23 = 100	2.26 = 100

¹*a* = variables with loading greater than the mean of the absolute loading in each principal component.

solubility and Ca^{2+} -ATPase activity had high contributions (23 - 27%) to the total variation followed by carbonyl content (17%). In PC2, myofibrillar protein surface hydrophobicity and sarcoplasmic protein solubility had high loadings (32 - 36%) followed by carbonyl content and reactive sulfhydryl content (13 - 16%).

One of the main reasons PCA was conducted in this study was to better interpret the data to show the influence of ultimate pH on biochemical properties of proteins in turkey meat. By plotting the object scores for PC1 and PC2, it can be seen how the objects are placed in the multivariate space (Figure 3.2). Figure 3.2A showed two clear and distinct groups (fresh and frozen meat) while Figure 3.2B showed at least 4 groups. By comparing the graphs in Figure 3.2, it was evident that PC1 (horizontal axis) was the main factor separating fresh and frozen samples in the multivariate space. On the other hand, PC2 tended to disperse the samples on the vertical axes and seemed to be more associated with ultimate pH rather than storage effect. As mentioned previously, sarcoplasmic protein solubility and myofibrillar protein surface hydrophobicity had the high loadings on the PC2. Thus, by observing at the right region of the graph reported in Figure 3.2B, two groups (1 and 2) can be differentiated. All the meat samples with high ultimate pH were in group 2 while the majority of the samples with low ultimate pH were located in group 1. Furthermore, 50% of the meat samples with normal pH fell in group 1 while 38% fell in group 2; the remaining 12% did not belong to any of the groups nor had characteristics similar to both low and high pH meat.

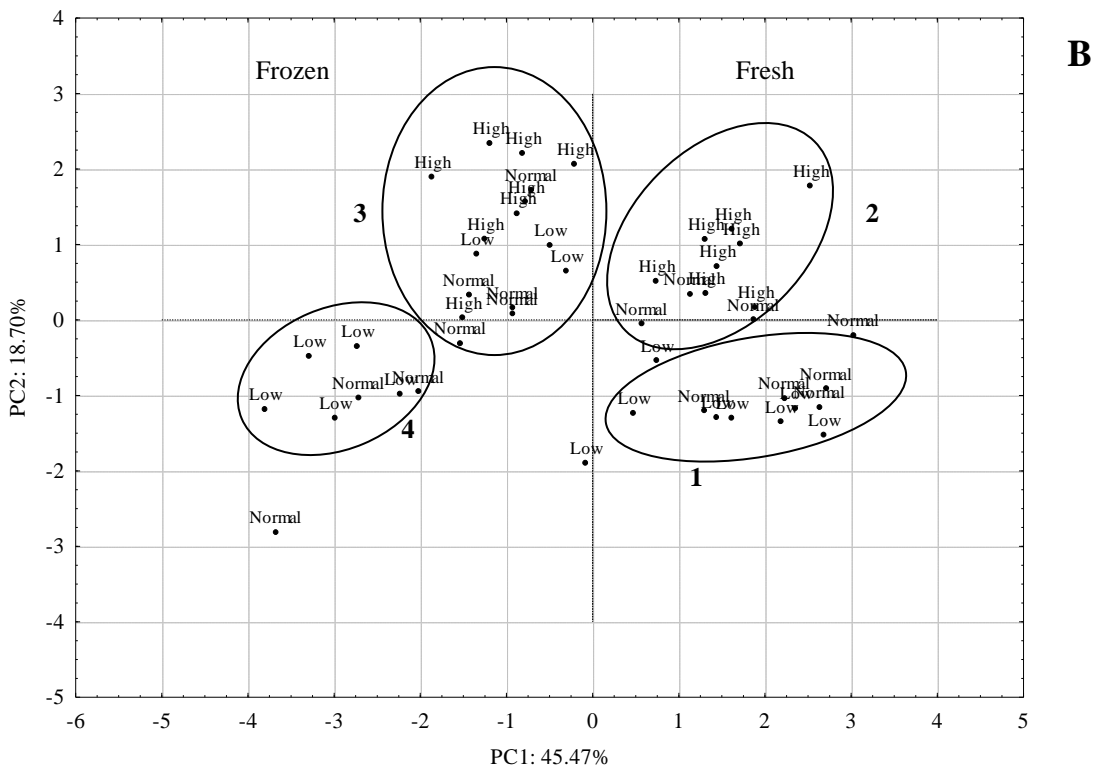
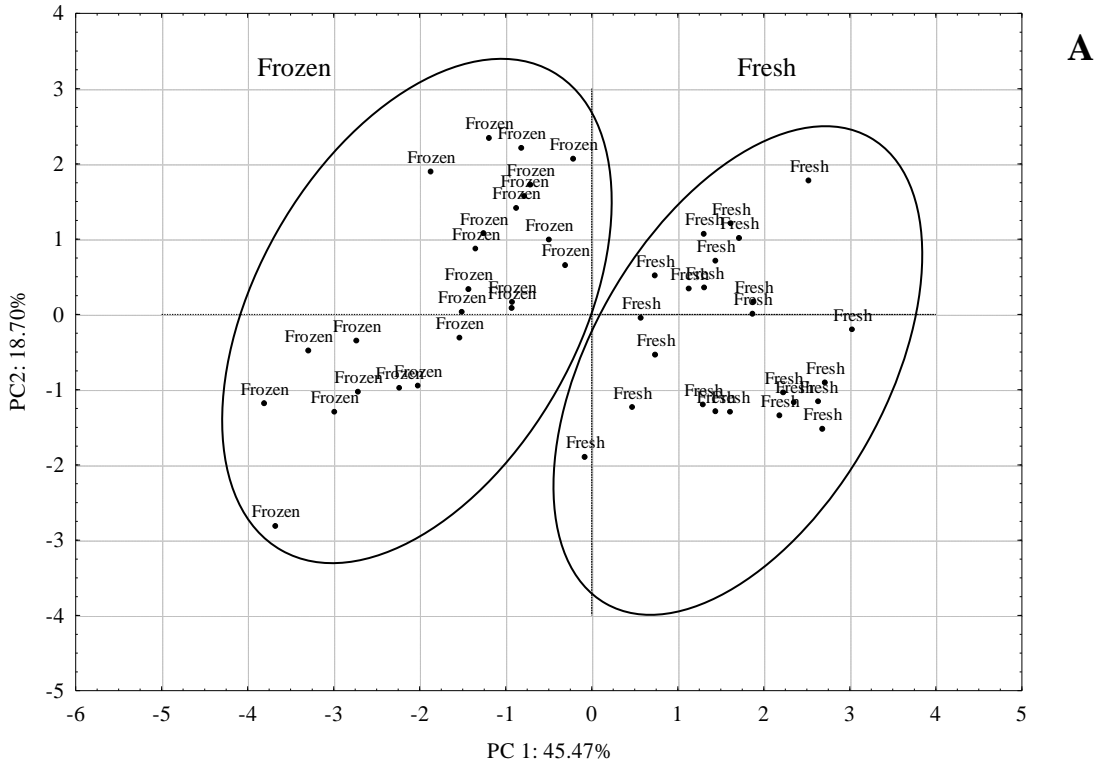


Figure 3.2. Score plot of the two principal components; A: Freezing, B: pH
 Low = low pH meat; Normal = normal pH meat; High = high pH meat.

In the left region of the graph reported in Figure 3.2B, where the frozen samples are located, the two groups appeared to be more unevenly distributed compared to the right region; 100, 63 and 37.5% of high, normal and low pH meat samples, respectively, fell in group 3. Interestingly, after frozen storage, a relevant part of the low pH meat samples were found in group 3 along with the majority of normal and high pH meat samples. This may indicate that the biochemical properties of protein of the three classes of meat tend to become similar after freezing. Group 4 contained the remaining meat samples which were predominantly low and normal pH meat samples.

In summary, results from PCA supported our previous findings obtained by univariate analysis that low and normal pH meat did not significantly differ by each other in terms of biochemical properties of proteins. Therefore, when searching for valid tools to discriminate the quality of poultry meat, other biochemical or biological characteristics needs to be evaluated; protein denaturation is not the sole factor contributing to formation of meat with low ultimate pH value.

3.4 CONCLUSION

Ultimate pH at 24 h *post mortem* and freezing had significant effects on the biochemical and functional properties of proteins in turkey breast meat. Low and normal pH meat showed similar properties whereas high pH meat was found to have higher protein solubility and hence expected to have better functional properties. Freezing caused denaturation and oxidation of proteins shown by the biochemical changes after frozen storage. Principal component analysis was

conducted and supported these findings. The main interesting discovery from this experiment was the similar extent of protein denaturation observed from low and normal pH meat which indicated that other factors (phospholipase A₂ (PLA₂) and/or protease enzyme activities and proteoglycans) may contribute to the reduced meat quality characteristic of low pH turkey meat. Furthermore, this study revealed that the biochemical properties of proteins of low, normal and high pH meat tend to become similar after freezing. Therefore, a thorough understanding of the biochemical and functional properties and their relationships will assist in developing strategies to improve meat quality and protein functionality.

3.5 REFERENCES

- Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. *Poult. Sci.* 76:1042-1046.
- Barbut, S. 1993. Color measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. *Food Res. Int.* 26:39-43.
- Barbut, S. 1996. Estimates and detection of the PSE problem in young turkey breast meat. *Can. J. Anim. Sci.* 76:455-457.
- Barbut, S., L. Zhang, and M. Marcone. 2005. Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poult. Sci.* 84:797-802.
- Benjakul, S. and F. Bauer. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. *J. Sci. Food Agric.* 80:1143-1150.
- Benjakul, S., W. Visessanguan, C. Thongkaew, and M. Tanaka. 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. *Food Res. Int.* 36:787-795.
- Betti, M., B. L. Schneider, W. V. Wismer, V. L. Carney, M. J. Zuidhof, and R. A. Renema. 2009. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poult. Sci.* 88:1085-1095.
- Bianchi, M., D. L. Fletcher, and D. P. Smith. 2005. Physical and functional properties of intact and ground pale broiler breast meat. *Poult. Sci.* 84:803-808.

- Binsi, P. K., B. A. Shamasundar, and A. O. Dileep. 2007. Physico-chemical and functional properties of proteins from green mussel (*Perna viridis*) during ice storage. *J. Sci. Food Agric.* 87:245-254.
- Careche, M., S. Cofrades, J. Carballo, and F. J. Colmenero. 1998. Emulsifying and gelation properties during freezing and frozen storage of hake, pork, and chicken actomyosins as affected by addition of formaldehyde. *J. Agric. Food Chem.* 46:813-819.
- Chen, T., G. Zhou, X. Xu, G. Zhao, and C. Li. 2010. Phospholipase A₂ and antioxidant enzyme activities in normal and PSE pork. *Meat Sci.* 84:143-146.
- Choi, Y. J. and J. W. Park. 2002. Acid-aided protein recovery from enzyme-rich Pacific Whiting. *J. Food Sci.* 67:2962-2967.
- Cofrades, S., M. Careche, J. Carballo, and F. J. Colmenero. 1996. Freezing and frozen storage of actomyosin from different species. *Z Leb. Unt. For.* 203:316-319.
- de Vries, A. G., L. Faucitano, A. Sosnicki, and G. S. Plastow. 2000. The use of gene technology for optimal development of pork meat quality. *Food Chem.* 69:397-405.
- Dileep, A. O., B. A. Shamasundar, P. K. Binsi, F. Badii, and N. K. Howell. 2005. Effect of ice storage on the physicochemical and dynamic viscoelastic properties of ribbonfish (*Trichiurus spp*) meat. *J. Food Sci.* 70:E537-E545.
- Farouk, M. M., K. J. Wieliczko, and I. Merts. 2003. Ultra-fast freezing and low storage temperatures are not necessary to maintain the functional properties of manufacturing beef. *Meat Sci.* 66:171-179.

- Fraqueza, M. J., A. S. Cardoso, M. C. Ferreira, and A. S. Barreto. 2006. Incidence of *Pectoralis major* turkey muscles with light and dark color in a Portuguese slaughterhouse. *Poult. Sci.* 85:1992-2000.
- Goonewardene, L. A., P. S. Mir, E. K. Okine, Z. Mir, and M. He. 2004. Study of carcass, organ, muscle, fat tissue weight, and concentration in rats fed CLA or its precursors by principal component analysis. *Can. J. Anim. Sci.* 84:537-543.
- Gornall, A. G., J. C. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751-766.
- Hayakawa, S. and S. Nakai. 1985. Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. *J. Food Sci.* 50:486-491.
- Karlsson, A. 1992. The use of principal component analysis (PCA) for evaluating results from pig meat quality measurements. *Meat Sci.* 31:423-433.
- Kim, Y. S., J. W. Park, and Y. J. Choi. 2003. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. *Fish. Sci.* 69:1231-1239.
- Kinsella, J. E. 1982. Relationship between structure and functional properties of food proteins. In *Food Proteins*. P. F. Fox and J. J. Condon, eds. Applied Science Publishers Ltd., USA.
- Li-Chan, E., S. Nakai, and D. F. Wood. 1985. Relationship between functional (fat binding, emulsifying) and physicochemical properties of muscle proteins. Effects of heating, freezing, pH and species. *J. Food Sci.* 50:1034-1040.

- Mercier, Y., P. Gatellier, M. Viau, H. Remington, and M. Renerre. 1998. Effect of fat and vitamin E on colour stability and lipid and protein oxidation in turkey meat during storage. *Meat Sci.* 48:301-318.
- Nakai, S. 1996. Food proteins: Properties and characterization. Page 199 in *Food Proteins: Properties and Characterization*. S. Nakai and H. W. Modler, eds. Springer, Berlin.
- Noguchi, S. and J. J. Matsumoto. 1970. Studies on the control of the denaturation of the fish muscle proteins during the frozen storage. 1. Preventive effect of Na-glutamate. *Bull. Jap. Soc. Sci. Fish.* 36:1078–1087.
- Offer, G. and P. Knight. 1988. The structural basis of water-holding in meat. Pages 173-243 in *Developments in Meat Science*. R. A. Lawrie, ed. Elsevier Applied Science, London, UK.
- Oliver, C. N., B. W. Ahn, E. J. Moerman, S. Goldstein, and E. R. Stadtman. 1987. Age-related changes in oxidized proteins. *J. Biol. Chem.* 262:5488-5491.
- Omana, D. A., Y. Xu, V. Moayed, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: chemical and functional properties of recovered proteins. *Proc. Biochem.* 45:375-381.
- Owen, F. R. 1996. Food Chemistry. Pages 14-16, 371 in *Food Chemistry*. 3rd ed. F. R. Owen, ed. Marcel Dekker, Inc., USA.
- Owens, C. M., E. M. Hirschler, S. R. McKee, R. Martinez-Dawson, and A. R. Sams. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. *Poult. Sci.* 79:553-558.

- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Pietrzak, M., M. L. Greaser, and A. A. Sosnicki. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. *J. Anim. Sci.* 75:2106-2116.
- Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. *Poult. Sci.* 80:676-680.
- Rowe, L. J., K. R. Maddock, S. M. Lonergan, and E. Huff-Lonergan. 2004. Influence of early postmortem protein oxidation on beef quality. *J. Anim. Sci.* 82:785-793.
- Smith, G. L. 1991. Principal component analysis: an introduction. *Anal. Proc.* 28:15-161.
- Smith, D. P. and J. K. Northcutt. 2009. Pale poultry muscle syndrome. *Poult. Sci.* 88:1493-1496.
- Soyer, A., B. Özalp, Ü. Dalmis, and V. Bilgin. 2010. Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. *Food Chem.* 120:1025-1030.
- Stadtman, E. R. 1990. Metal ion-catalyzed oxidation of proteins: Biochemical mechanism and biological consequences. *Free Rad. Biol. Med.* 9:315-325.
- Strasburg, G. M. and W. Chiang. 2009. Pale, soft, exudative turkey - The role of ryanodine receptor variation in meat quality. *Poult. Sci.* 88:1497-1505.

- Tausky, H. H. and E. Shorr. 1952. A microcolorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* 202:675-685.
- Van Laack, R. L. J. M. and J. L. Lane. 2000. Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. *Poult. Sci.* 79:105-109.
- Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* 79:1057-1061.
- Velleman, S. G. 1998. What's outside the cell and how does it affect avian bone and muscle formation? Pages 71-88. U.S.A.
<http://www.poultryscience.org/pba/1952-2003/1998/1998%20Velleman.pdf>
- Wang, R. R., X. J. Pan, and Z. Q. Peng. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of *pectoralis majors* in broilers. *Poult. Sci.* 88:1078-1084.
- Warriss, P. D. 2010. Meat Science: An introductory text. Pages 68-71 in *Meat Science: An Introductory Text*. 2nd ed. P. D. Warriss, ed. CABI Publishing, USA.
- Wilhelm, A. E., M. B. Maganhini, F. J. Hernandez-Blazquez, E. I. Ida, and M. Shimokomaki. 2010. Protease activity and the ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. *Food Chem.* 119:1201-1204.
- Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poult. Sci.* 81:579-584.

- Xia, X., B. Kong, Q. Liu, and J. Liu. 2009. Physicochemical change and protein oxidation in porcine *longissimus dorsi* as influenced by different freeze-thaw cycles. *Meat Sci.* 83: 239-245.
- Xiong, Y. L. 2000. Protein oxidation and implication for muscle food quality. Pages 85-111 in *Antioxidants in Muscle Foods*. E. A. Decker, C. L. Faustman, and C. J. Lopez-Bote, eds. John Wiley and Sons Inc., NY, USA.
- Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.

CHAPTER 4. FUNCTIONAL AND RHEOLOGICAL PROPERTIES OF PROTEINS IN FROZEN TURKEY BREAST MEAT WITH DIFFERENT ULTIMATE pH³

4.1 INTRODUCTION

The poultry market has increased turkey sales from whole birds to further processed products over the past 25 years (National Chicken Council, 2009). The world poultry meat production is projected to rise by 2.3% in 2015 as consumers continue to increase their demand for more convenient, ready to eat products (Poultry Marketplace, 2010). Functionality of meat proteins plays an important role in the production of further processed products. In general, pH of meat has important influence on the functional properties of proteins (Owen, 1996), such as water holding capacity (WHC), emulsifying and foaming properties and gel forming ability. Myofibrillar and sarcoplasmic proteins in meat are effective emulsifiers of fat in food systems such as sausages. However, myofibrillar proteins form more stable emulsions than sarcoplasmic proteins (Ramachandran et al., 2007). Textural properties are also important in further processed products because it affects consumer acceptability.

Turkey with pale, soft, exudative (PSE)-like characteristics is one of the growing concerns in the poultry industry as it affects meat quality. The PSE condition was first observed in the pork industry. In pigs, the combination of rapid *post mortem* pH decline and high carcass temperature leads to protein denaturation in the muscle (Santos et al., 1994; Guardia et al., 2005). Extensive

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research has indicated that a major proportion of PSE had a genetic basis that was directly related to porcine stress syndrome (PSS) and malignant hyperthermia. This was shown to be due to a major gene effect of a mutation in the pig ryanodine receptor (RYR), a protein involved in the regulation of calcium metabolism in muscle (Fujii et al., 1991). Strasburg and Chiang (2009) also hypothesized that differences in ryanodine receptor activities in turkey could lead to altered calcium homeostasis and eventually to the development of a PSE-like condition. In a recent study, Ziober et al. (2010) concluded that the expression pattern of RYR transcripts in broiler chickens was altered by heat stress and halothane treatment. Even though this has been linked to PSE-like condition in poultry, the authors measured only color (lightness (L^*) values) rather than pH or any other indicators of functionality. However, color alone is not the most useful indicator of a PSE-like condition in poultry (Fraqueza et al., 2006). Thus, the biochemical mechanism observed in pigs has not been fully confirmed in poultry. Generally, it is believed that turkey and broiler chickens behave differently from pigs in regard to PSE expression due to genetic differences between mammalian and avian species (Smith and Northcutt, 2009). Improper processing, such as delay in chilling (Rathgeber et al., 1999) or of artificially created conditions, such as inducing fast drop of pH in turkey breast muscle that were kept at higher chiller temperatures (McKee and Sams, 1998; Alvarado and Sams, 2004) may also result in PSE-like condition. However, several studies seem to indicate that the condition in poultry is more related to the extent of acidification (i.e. low pH at 24 h) rather than fast drop in pH immediately after slaughter (Wilkins et al., 2000;

Petracci et al., 2004; Fraqueza et al., 2006). Other factors which influence the development of PSE-like poultry meat include season, environment, heat exposure, and pre-slaughter handling practices (Owens et al., 2009; Wang et al., 2009).

The loss of protein functionality in meat with low pH₂₄ may have serious consequences as it reduces suitability for the production of further processed products. Conversely, dark, firm, dry (DFD) meat has higher pH than normal meat, shorter shelf life, and darker appearance, which affects the color of processed products and consumer acceptability (Allen et al., 1997; Guardia et al., 2005). It has been estimated that the turkey industry could be losing at least U.S. \$200 million per year due to reduced protein functionality in further processed products (Owens et al., 2009). Currently, details on the characteristics and occurrences of DFD-like turkey meat are not available. As the turkey industry increases the production of further processed products, such as formed breast loaves and rolls, there is a need for processors and industry to find ways to optimize the use of meat with different ultimate pH taking into account differences in protein functionality.

It is generally hypothesized that ultimate pH leads to different extent of protein denaturation, which ultimately affects functional properties of proteins. It has been shown that chicken and turkey with low pH had reduced protein functionality, such as decreased WHC and weaker gel formation (Barbut, 1997; Zhang and Barbut, 2005). However, studies conducted by Van Laack and Lane (2000) and Van Laack et al. (2000) showed that avian myofibrillar proteins are

relatively resistant to *post mortem* protein denaturation in contrast to mammalian proteins which indicates that factors other than protein denaturation are responsible for the low WHC observed in PSE-like chicken breast meat; other biochemical mechanisms may be involved (Molette et al., 2003). Furthermore, the main findings from Chapter 3 have shown that low and normal pH turkey breast meat have similar extent of protein denaturation, while low pH meat had lower WHC, which reveals that protein denaturation may not be directly associated with water retention ability. The study has also shown that after frozen storage, the biochemical properties of proteins in low, normal, and high pH meat tend to become very similar; therefore, since the functionality of proteins depend mostly on their biochemical characteristics, this work has been carried out to study the effect of ultimate pH at 24 h *post mortem* on various functional properties of proteins in frozen turkey breast meat.

4.2 MATERIALS AND METHODS

4.2.1 Sample selection

A total number of 48 skinless, boneless breast fillets from Hybrid Tom turkeys were initially selected from a local processing plant (Lilydale Inc., Edmonton, Alberta, Canada) at 24 h *post mortem*, with 16 per color group (pale, normal, dark). Selection was made using color measurement based on lightness (L^*) values as reported by Zhang and Barbut (2005). The average turkey age was 116 days and the average flock weight was 14.3 kg. The samples were labeled and placed individually in plastic bags, packed on ice and transported to the laboratory. L^* values and pH_{24} were measured again on all 48 breast samples in

the laboratory. Eight breast samples were further selected within each class (pale, normal, and dark), according to their ultimate pH at 24 h *post mortem* (pH₂₄). In this experiment, extremely low and extremely high pH meat was selected because ultimate pH has been indicated as a valid tool to differentiate protein functionality. The average L* and pH values of the samples were within the following range: pale (L* > 52, pH ≤ 5.7), normal (46 < L* < 52, 5.9 < pH < 6.1), and dark (L* < 46, pH ≥ 6.3) and referred to as low, normal, and high pH meat, respectively. Each breast was labeled according to the class and was minced individually in a Kitchen-Aid food processor (Model KFP 7500B, KitchenAid, St. Joseph, MI, USA) for 2 min. The food processor was pre-chilled and operated in a cold room (4°C) to prevent the temperature of the samples from exceeding 10 °C throughout the mixing process. All the analyses were carried out in frozen meat samples that were vacuum packaged in polyethylene bags and stored at -30°C for 3 weeks and thawed overnight at 4°C.

4.2.2 Color measurement

The methodology for color measurement is described in Chapter 3 (refer to section 3.2.2).

4.2.3 pH measurement

The methodology for pH measurement is described in Chapter 3 (refer to section 3.2.3).

4.2.4 Emulsion activity index (EAI) and emulsion stability index (ESI)

The ability of the sarcoplasmic and myofibrillar protein fractions to form emulsions was determined as emulsion activity index (EAI) according to the

method described by Pearce and Kinsella (1978) as per the modifications of Moure et al. (2002). Sarcoplasmic protein solution was prepared by homogenizing 2 g of meat with 40 mL of 0.03 M phosphate buffer (pH 7.4). Homogenates were centrifuged using Avanti J-E refrigerated centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) at 15,300 x g for 15 min at 4°C and filtered through Whatman No. 1 filter paper. The supernatant obtained after filtration was used as sarcoplasmic protein solution. Myofibrillar protein solution was prepared after separation of sarcoplasmic proteins. The sediment was further homogenized with 40 mL of 0.05 M phosphate buffer containing 0.55 M KI (pH 7.4). Homogenates were centrifuged at 15,300 x g for 15 minutes at 4°C and filtered through Whatman No.1 filter paper. The supernatant obtained after filtration was used as myofibrillar protein solution. The protein concentration of the supernatants was assessed using the Biuret procedure (Gornall et al., 1949) with bovine serum albumin (BSA) as standard. Known volumes of protein at different concentrations were prepared and mixed with corn oil in the ratio 3:1 using a homogenizer (Fisher Scientific, ON, Canada) at 13,500 rpm for 1 min. Immediately after homogenization, aliquots of 50 µl of the emulsion were diluted to 5 mL with 0.1% sodium dodecyl sulphate (SDS) solution. The turbidity of the emulsion was measured at 500 nm using a spectrophotometer (V-530, Jasco Corporation, Japan). EAI was measured as initial absorbance and was calculated from the equation (Selmane et al., 2008):

$$\text{EAI} = 2.33 \times A_0$$

where A_0 is the absorbance measured immediately after emulsion preparation.

The emulsion stability index (ESI) was determined by measuring the absorbance of the emulsion after 10 min. ESI was calculated from the equation (Selmane et al., 2008):

$$\text{ESI} = 10 \times \left(\frac{A_0}{(A_0 - A_{10})} \right)$$

where A_{10} is the absorbance measured after 10 min. ESI measures the rate of decrease of emulsion turbidity due to droplet coalescence and creaming, which leads to emulsion destabilization. EAI and ESI increases when proteins favour emulsion formation and stabilization, respectively (Selmane et al., 2008).

4.2.5 Foamability and foam stability

The foamability of the extracted sarcoplasmic and myofibrillar protein fractions was determined by the method of Wild and Clark (1996). Protein solutions were prepared as described in section 3.2.4. Known volumes of protein at different concentrations were whipped in a vortex mixer (Fisher Scientific, ON, Canada) at speed 10 for 1 min. Foamability or foam expansion (FE) was expressed as percentage volume increase after mixing using the equation:

$$\text{Foam expansion (\% FE)} = \frac{\text{Foam volume (mL)}}{\text{Initial liquid volume}} \times 100$$

The stability of the foam or foam stability (FS) was calculated as the percentage of foam remaining after 30 min at 25°C using the equation:

$$\text{Foam stability (\% FS)} = \frac{\text{Volume of foam (mL) retained after 30 min}}{\text{Volume of foam after whipping}} \times 100$$

4.2.6 Cooking loss

Cooking loss of samples was determined by the method of Zhang and Barbut (2005). Approximately 50 g of meat (W1) was mixed with 2.5% sodium chloride and packed in 50 mL polypropylene centrifuge tubes. The tubes were centrifuged (Beckman Coulter, Inc., Palo Alto, CA, USA) at 250 rpm for 30 s to pack the meat. The tubes were then heated at 95°C in a water bath until the internal temperature of the samples reached 75°C, at which the samples are considered as cooked. The temperature was checked using thermocouples inserted in the center of the samples. After cooking, the tubes were cooled at room temperature for 15 min and the meat was weighed again (W2) to determine the loss in weight during cooking.

$$\text{Cooking loss (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

4.2.7 Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out (Laemmli, 1970) using 10-20% ready gels (Bio-Rad Laboratories, Inc., Hercules, CA) at a constant voltage mode (200 V) in a Mini-PROTEAN tetra cell attached to a PowerPac Basic electrophoresis apparatus (Bio-Rad Laboratories Inc., 1000 Alfred Nobel Drive, Hercules, California, USA). 20 µg of protein were loaded for all samples. Protein markers of high range molecular weight obtained from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA) were loaded into a separate well for comparison of molecular weights. Gels after staining and destaining were scanned using an Alpha Innotech

gel scanner (Alpha Innotech Corp., San Leandro, CA) with FluorChem SP software.

4.2.8 Texture profile analysis

Texture profile analysis (TPA) was carried out by the method of Omana et al. (2010) using a texture profile analyzer (TA-XT Express, Stable micro systems, Ltd., Surrey, England) under TPA test mode. Gels were prepared as described in section 3.2.6. The samples were kept overnight at 4°C and cut into cylindrical shapes (1.0 cm-height and 4.0 cm-diameter) for TPA. A double compression cycle test was performed up to 50% compression of the original height with an aluminum cylinder probe of 5 cm diameter. The elapse time between the two compression cycles was 1 s. The trigger force used for the test was 5 g with a test speed of 5 mm/s. When the test was completed, the software (TA-XT Express Software) calculated values for hardness (maximum force required to compress the sample), springiness (ability of the sample to recover its original form after deforming force was removed), chewiness (work needed to chew a solid sample to a steady state of swallowing), cohesiveness (extent to which the sample could be deformed prior to rupture), and resilience (ability of the sample to regain its original position after compression).

4.2.9 Gel strength

Gel strength was conducted by the method of Nowsad et al. (2000). Gels were prepared as described in section 3.2.6 to determine gel strength and folding ability. The samples were kept overnight at 4°C and were cut into cylindrical shapes (2.5 cm-height and 4.0 cm-diameter). Using the texture profile analyzer

(TA-XT Express, Stable micro systems, Ltd., Surrey, England) equipped with a ball probe (half inch diameter), in cycle test mode, the samples were tested at a speed of 5 mm/s. Gel strength was expressed independently as breaking strength (g) and deformation (cm).

Folding ability was determined by folding a 2-mm thick gel disc sample into halves and quarters (Nowsad et al. 2000). The scales used were: a = no crack when folded into quarter, b = no crack when folded into half but crack when folded into quarter; c = crack when folded into half, d = broke and split into halves.

4.2.10 Dynamic viscoelastic behavior

Dynamic viscoelastic behavior (DVB) of raw meat samples was carried out by the method of Omana et al. (2010) with modifications using a Physica MCR Rheometer (Anton Paar GmbH, Virginia, USA) equipped with a 2.5 cm parallel plate measuring geometry, in the temperature range of 6.89°C to 80.00°C (heating) and 80.00°C to 6.89°C (cooling), under oscillatory mode. A fine paste was made by grinding approximately 4 g of raw meat with 2.5% sodium chloride and was used for DVB measurement. The gap between the measuring geometry and the peltier plate was adjusted to 1000 μm . The measurements were made under controlled strain (0.5%) with a frequency of 1.0 Hz. Linear viscoelastic region (LVR) was determined using amplitude sweep in a range of deformation from 0.1 to 10%. The heating and cooling rate used was 2°C/min. The results of the measurements were expressed as the storage modulus (G') and loss modulus

(G"). The ratio of these two values, tan delta ($\tan \delta$), was also recorded throughout the heating and cooling processes.

4.2.11 Statistical analysis

Each assay was carried out in 8 replications per meat type and was done at least in duplicate. Reported results represent an average of each experimental assay. The data were analyzed using One-way ANOVA and the Mixed procedure of SAS (SAS version 9.0, SAS Institute, Cary, NC, USA, 2006). The model tested the main effects for meat pH group (low, normal, and high). Differences between group means were determined using HSD Tukey differences and were reported as significant at the $P < 0.05$ level.

4.3 RESULTS AND DISCUSSION

4.3.1 Emulsifying properties

Emulsion is defined as a heterogeneous system which consists of at least two immiscible liquid phases, with one dispersed in the other, in the form of droplets (Das and Kinsella, 1990). Stabilization of emulsions is achieved by the interaction of the protein matrix with fat within the sample and the physical entrapment of fat globules via protein-protein interactions, which leads to the formation of an interfacial protein layer that surrounds and stabilizes the fat globules (Barbut, 1995). The emulsion activity index (EAI) measures the ability of proteins to rapidly adsorb at the water-oil interface during the formation of an emulsion to prevent flocculation and coalescence. In this study, emulsifying properties of extracted sarcoplasmic and myofibrillar proteins were studied since the data for such parameters as a function of ultimate pH is scarce. EAI of

sarcoplasmic proteins at 1.5 and 3.0 mg/ml protein concentration was significantly higher ($P < 0.05$, $P < 0.0001$, respectively) in the fraction from high pH meat compared to low pH meat (Table 4.1). Qiao et al. (2001) found that high pH broiler breast meat had higher emulsification capacity compared to that of low pH meat. Emulsifying properties are related to the hydrophobic-hydrophilic balance of proteins. The increase in EAI of sarcoplasmic proteins with different concentrations from high pH meat may be due to the increased solubility of proteins or higher sulfhydryl content as reported in Chapter 3. Mohan et al. (2006) reported an increase in EAI with increase in reactive sulfhydryl groups in fish proteins. In the case of myofibrillar proteins, there was no significant difference ($P > 0.05$) in EAI observed for both protein concentrations for all three classes of meat.

The emulsion stability index (ESI) measures the rate of emulsion destabilization over a period of time due to droplet coalescence and creaming. In the case of sarcoplasmic proteins at 1.5 mg/ml protein concentration, there was no significant difference ($P > 0.05$) in ESI for any of the samples (Table 4.1). However, at 3.0 mg/ml protein concentration, the ESI for low pH and normal pH meat was 15.8 and 17.2, respectively, and was not statistically different. For myofibrillar proteins, there was no significant difference ($P > 0.05$) in ESI for any sample. Myofibrillar proteins are important in forming interfacial films to encapsulate fat particles producing stable emulsions. Mohan et al. (2006) suggested that emulsion stability of myofibrillar proteins from fish was found to be concentration dependent. However, in this study, it was found that the stability

Table 4.1. Changes in emulsion activity index (EAI) and emulsion stability index (ESI) of sarcoplasmic and myofibrillar proteins in low, normal, and high pH meat¹

Classes of Meat	Emulsion activity index				Emulsion stability index			
	Sarcoplasmic proteins		Myofibrillar proteins		Sarcoplasmic proteins		Myofibrillar proteins	
	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml
Low pH	1.54 ± 0.28 ^b	2.05 ± 0.22 ^b	1.65 ± 0.26	2.42 ± 0.30	14.8 ± 2.3	15.8 ± 1.1 ^{a,b}	19.2 ± 2.0	19.7 ± 2.2
Normal pH	1.77 ± 0.23 ^{a,b}	2.20 ± 0.22 ^b	1.73 ± 0.18	2.57 ± 0.53	15.7 ± 2.1	17.2 ± 2.0 ^a	18.6 ± 2.7	19.2 ± 0.6
High pH	2.01 ± 0.33 ^a	2.69 ± 0.28 ^a	1.88 ± 0.44	2.82 ± 0.20	16.0 ± 1.6	15.0 ± 0.8 ^b	18.3 ± 1.2	18.4 ± 2.4
Source of Variation (P - Value)	0.0140	<0.0001	0.3295	0.1528	0.4639	0.0282	0.6794	0.4586

¹ Results are presented as means ± standard deviations (n=8). Dissimilar superscripts in the same column denote significant difference ($P < 0.05$).

of emulsion for myofibrillar proteins at both concentrations was relatively similar, suggesting that emulsion stability was independent of protein concentration in the case of turkey meat proteins (at least at the two concentrations tested in this study).

This finding regarding extracted myofibrillar proteins from frozen turkey meat samples is quite interesting; emulsion characteristics of proteins depend mostly on their denaturation level, and thus these results supported our previous results in which freezing tend to lead to the same level of protein denaturation independently of ultimate pH (Chapter 3).

4.3.2 Foaming properties

Foamability is an important characteristic of proteins in which they act as surfactants and form a flexible cohesive film that surrounds air bubbles. Foamability is desirable in many foam based products, such as cakes, meringues, and mousses. Although foam properties are not much relevant in the meat industry, foamability is dependent upon the rate of protein denaturation (Hughes and Baxter, 2001), which reveals the quality of proteins. Proteins that rapidly unfold and adsorb at the air-liquid interface during bubbling or whipping exhibit enhanced foamability (Nakai, 1996). The foam expansion of sarcoplasmic proteins at 1.5 mg/ml protein concentration was significantly higher ($P < 0.05$) in high pH meat compared to low pH meat (Table 4.2). The results in Chapter 3 showed that high pH meat had higher protein solubility, hence increasing the amount of proteins adsorbing at the air-liquid interface and thus improving foam formation (Tsaliki et al., 2002). However, there was no significant difference

Table 4.2. Changes in foam expansion (%) and foam stability (%) of sarcoplasmic and myofibrillar proteins in low, normal, and high pH meat¹

Classes of Meat	Foam expansion (%)				Foam stability (%)			
	Sarcoplasmic proteins		Myofibrillar proteins		Sarcoplasmic proteins		Myofibrillar proteins	
	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml	1.5 mg/ml	3.0 mg/ml
Low pH	50.0 ± 21.5 ^b	68.1 ± 22.5	110.0 ± 44.2	135.6 ± 34.2	63.6 ± 11.6	68.9 ± 10.9	71.5 ± 4.4	72.7 ± 77.7 ^a
Normal pH	55.0 ± 22.7 ^{a,b}	83.1 ± 35.8	115.6 ± 32.9	135.6 ± 43.7	59.2 ± 13.3	62.4 ± 2.3	66.7 ± 11.2	59.6 ± 79.0 ^b
High pH	81.3 ± 30.7 ^a	102.5 ± 34.3	136.9 ± 17.1	157.5 ± 19.1	62.2 ± 10.5	61.0 ± 7.5	65.2 ± 8.4	66.8 ± 77.5 ^{a,b}
Source of Variation (P - Value)	0.0479	0.1147	0.2577	0.3474	0.7591	0.1309	0.3255	0.0235

¹ Results are presented as means ± standard deviations (n=8). Dissimilar superscripts in the same column denote significant difference ($P < 0.05$).

($P > 0.05$) in foam expansion of sarcoplasmic proteins at 3.0 mg/ml protein concentration. Ultimate pH had no significant effect ($P > 0.05$) on foam expansion of myofibrillar proteins at 1.5 and 3.0 mg/ml protein concentration. Townsend and Nakai (1983) found a correlation between protein surface hydrophobicity values and foaming characteristics; it was reported that protein surface hydrophobicity values above 700 usually result in good foaming properties due to the flexible protein conformation. The protein surface hydrophobicity of myofibrillar proteins in low, normal, and high pH meat was close to 700 (Chapter 3), indicating good foaming properties of turkey breast meat proteins irrespective of the pH₂₄ of the meat.

Foam stability measures the ability of proteins to stabilize these foams. Ultimate pH had no significant effect ($P > 0.05$) on foam stability of sarcoplasmic proteins at 1.5 and 3.0 mg/ml protein concentration (Table 4.2). At 1.5 mg/ml protein concentration, there was no significant difference on foam stability of myofibrillar proteins in the three classes of meat. However, foam stability for myofibrillar proteins at 3.0 mg/ml protein concentration was significantly higher ($P < 0.05$) for low pH meat compared to that of normal pH meat. This may be due to the higher myofibrillar protein hydrophobicity of low pH meat compared to that of normal pH meat (Chapter 3). Foam stability depends on the proper balance of flexibility and rigidity of proteins at the air-water interface and the ability to form the cohesive film with high resistance to shear deformation (Ramachandran et al., 2007).

4.3.3 Cooking loss

Water holding capacity (WHC) is an important property which determines meat quality and is used to evaluate PSE meat (Woelfel et al., 2002). Expressible moisture and cooking loss are good indicators of water holding capacity. The findings in Chapter 3 showed that the expressible moisture of normal and high pH meat (18.2 and 13.9%, respectively) was not statistically different. However, expressible moisture of low pH meat (23.5%) was significantly higher than that of normal and high pH meat. This was consistent with previous work which demonstrated that the expressible moisture from pale turkey and broiler breast meat was significantly higher than that of normal samples (Owens et al., 2000; Woelfel et al., 2002). It has been reported by several authors that lower muscle pH is associated with lower WHC, as evident in pale turkey meat (Barbut, 1993; Fraqueza et al., 2006).

Cooking loss showed a similar trend as for expressible moisture in Chapter 3. It was found that low pH meat had the highest cooking loss (9.8%), and was significantly higher ($P < 0.0001$) than that of normal (6.1%) and high pH meat (2.0%) (Table 4.3). Zhang and Barbut (2005) also reported a similar trend in chicken breast meat, in which cooking loss was highest for PSE (9.28%), compared to normal (5.90%) and DFD (3.09%) samples. Myofibrillar proteins in low pH meat are near the isoelectric point (pI), pH approximately 5.3 (Offer and Knight, 1988). In general, when myofibrillar proteins reach their pI, they lose the ability to bind water because actin and myosin molecules pack more closely together (Huff-Lonergan and Lonergan, 2005). However, low and normal pH meat had the same amount of protein denaturation according to protein solubility

Table 4.3. Cooking loss, gel strength, and textural characteristics of low, normal, and high pH meat¹

Measurements	Classes of Meat			Source of Variation (P - Value)
	Low pH meat	Normal pH meat	High pH meat	
Cooking loss (%)	9.8 ± 0.2 ^a	6.1 ± 1.1 ^b	2.0 ± 1.1 ^c	<0.0001
Breaking strength (g)	358 ± 18 ^c	413 ± 50 ^b	536 ± 48 ^a	<0.0001
Deformation (cm)	1.41 ± 0.21 ^b	1.39 ± 0.24 ^b	1.83 ± 0.14 ^a	0.0003
Folding test	c	c	a	
Texture profile analysis				
Hardness (N)	16.6 ± 1.6 ^b	21.1 ± 3.1 ^a	22.6 ± 4.1 ^a	0.0052
Springiness	0.86 ± 0.01 ^b	0.95 ± 0.06 ^a	0.96 ± 0.02 ^a	0.0002
Chewiness	979 ± 141 ^b	1369 ± 268 ^a	1561 ± 334 ^a	0.0016
Cohesiveness	0.66 ± 0.03	0.66 ± 0.03	0.69 ± 0.01	0.0980
Resilience	0.42 ± 0.03	0.43 ± 0.02	0.45 ± 0.01	0.0601

¹ Results are presented as means ± standard deviations (n=8). Dissimilar superscripts in the same row denote significant difference ($P < 0.05$).

(Chapter 3). This may indicate that surface polarity and/or hydrophobicity ratio of the proteins was the same (Kinsella, 1981) for low and normal pH meat resulting in the same amount of protein solubilised by the buffer solution used during the extraction. Hence, factors other than protein denaturation may play a role in influencing the lower water retention ability of low pH meat (Van Laack and Lane, 2000). Velleman (1998, 2000) suggested that modifications in the muscle structure involving proteoglycans, which are present in the extracellular matrix,

may be of importance in determining the WHC of PSE meat. Another possible explanation for lower WHC in poultry meat may be based on phospholipase A₂ (PLA₂) enzyme activity in muscle. PLA₂ enzymes cleave fatty acids in the sn-2 position of phospholipids to yield free fatty acids and lysophospholipids. Excessive PLA₂ activity causes increased membrane blebbing in mitochondria and in the sarcoplasmic reticulum which induces an excessive release of Ca²⁺ ions in the sarcoplasm. PLA₂ activity is also involved in the disruption of muscle cell membrane (sarcolemma) which contributes to exudative loss from meat. Recent studies conducted in poultry and pigs (Soares et al., 2003; Chen et al., 2010) confirmed that low *post mortem* pH values are associated with increased PLA₂ activity. Furthermore, elevated Ca²⁺ concentration in the cytoplasm may increase protease activity (i.e. calpain) affecting integrity of muscle fiber structure and thus impairing meat protein functionality (Wilhelm et al., 2010).

4.3.4 Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE profile of total proteins extracted from low, normal, and high pH meat is shown in Figure 4.1. Myosin and actin are the major proteins which contribute to most of the functional properties of myofibrillar proteins. Actin is not degraded by proteolytic enzymes and is resistant to denaturation (Van Laack et al., 2000). The intensity of bands corresponding to myosin heavy chain and actin did not show any difference in total proteins extracted from the three classes of meat. However, the protein bands in the lower molecular region at approximately 20 kDa, which correspond to Troponin I (Figure 4.1), were less

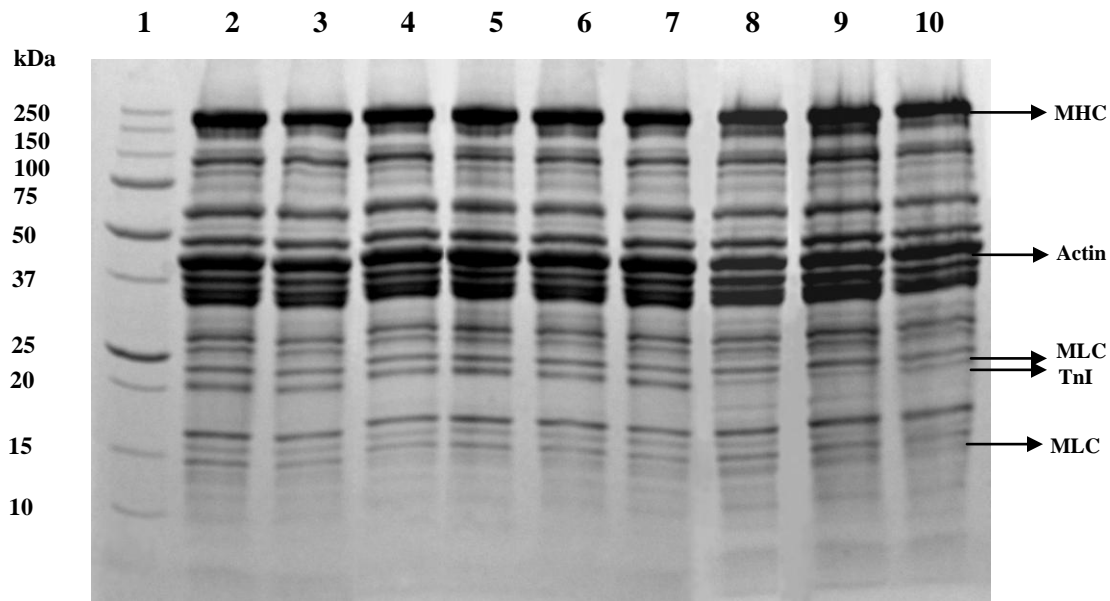


Figure 4.1. SDS-PAGE profile of total proteins extracted from low, normal, and high pH turkey breast meat. Lane 1: standard marker; lane 2-4: low pH meat from different samples; lane 5-7: normal pH meat from different samples; lane 8-10: high pH meat from different samples. MHC: Myosin heavy chains; MLC: Myosin light chains; TnI: Troponin I.

intense for high pH meat. Xiong and Brekke (1991) found that chicken breast myofibrils extracted at low pH (pH = 5.70) had a greater concentration of troponin I than the ones extracted at higher pH (pH = 6.00 or 6.50). It has been reported by Yamamoto and Maruyama (1973) that troponin I binds directly to tropomyosin and thus, it may be possible that at lower pH, troponin I dissociates from tropomyosin, yielding a more intense band. The same authors also reported that the troponin I-tropomyosin complex is influenced by the ionic strength. Another possible explanation may be due to some different genetic patterns between birds that develop high pH compared to the ones which develop low and normal pH. However, electrophoretic band patterns of proteins from low and

normal pH meat were similar. This further confirmed that the extent of protein denaturation in low and normal pH meat was the same.

4.3.5 Textural characteristics

Textural characteristics are important in the production of further processed meat products because they affect consumer acceptability. Texture profile analysis of low, normal, and high pH meat is presented in Table 4.3. Springiness is the ability of a sample to recover its original form after deformation and chewiness is the work needed to chew a sample to a steady state of swallowing (Martinez et al., 2004). Springiness and chewiness gives an indication of juiciness and tenderness, respectively (Caine et al., 2003). Hardness, springiness, and chewiness values of low pH meat were 16.6 N, 0.86, and 979, respectively, and were significantly lower ($P < 0.05$) than that of normal and high pH meat. The formation of an elastic gel depends on the water binding ability of proteins. During cooking of gels, low pH meat had higher cooking loss, thus adversely affecting textural characteristics. The low textural values in low pH meat may also be related to changes in muscle structure and/or enzymatic activities as mentioned in section 4.3.3. Zhang and Barbut (2005) observed that PSE chicken breast meat also had lower hardness values compared to normal and DFD samples. However, there was no significant difference ($P > 0.05$) in cohesiveness and resilience in the three classes of meat.

An interesting consideration resulting from the interpretation of these results was that low pH meat, minced and mixed with salt, resulted in a softer texture. From the consumer acceptance point of view, this may be a positive

rather than a negative aspect. However, to further understand consumer acceptance in terms of textural properties of products, a complete sensory panel evaluation is needed.

4.3.6 Gel strength

Gel strength analysis is widely used in the food industry and is a good indicator of elasticity of food products. Factors influencing gel forming ability are pH, ionic strength, protein solubility, and the type of extractable proteins (Lanier, 1986). Myofibrillar proteins play an important role in the gel properties of meat products because they influence textural and sensory properties. Moisture content of the three classes of meat used for gel strength analysis was approximately 75% and was not statistically different as a function of pH₂₄. High pH meat had the highest ($P < 0.0001$) breaking strength (536 g), followed by normal (413 g) and low (358 g) pH meat (Table 4.3), which indicates that high pH meat had better gel strength. The results from Chapter 3 showed that low and normal pH meat had the same extent of protein denaturation and was more than that of high pH meat as shown by protein solubility. This may be the reason for their lower gel strength compared to high pH meat. Alvarado and Sams (2004) demonstrated that protein denaturation and a decrease in myofibrillar protein solubility reduces the strength of heat-induced gels. Water binding ability of proteins also contributes to the formation of an elastic gel (Barbut, 1993). Decrease in gel strength was observed with an increase in cooking loss of turkey breast meat (Barbut, 1993). During cooking, high pH meat had the least cooking loss; hence it had greater ability to entrap water. This leads to the formation of a rigid gel matrix with increased gel

strength. Deformation is an indicator of cohesiveness. In this study, high pH meat was found to have a significantly higher ($P = 0.0003$) deformation (1.83 cm) compared to that of normal (1.39 cm) and low pH meat (1.41 cm), demonstrating greater ability to withstand deformation.

A folding test was conducted to indicate gel springiness (Nowsad et al., 2000). Folding test scores showed the same trend as in gel strength (Table 4.3). The scores for low and normal pH meat were 'c', which revealed that the samples cracked when folded into halves. However, high pH meat had the highest score, 'a', which showed that samples had no crack when folded into quarters. This confirmed that high pH meat had better gel properties compared to low and normal pH meat.

4.3.7 Rheological characteristics

Small strain test or dynamic rheological test has been widely used to study the heat-induced gelation of myofibrillar proteins (Hamann, 1988). Rheograms (heating and cooling) obtained for the three classes of meat are presented in Figure 4.2 and 4.3, respectively. The storage modulus (G') is a measure of the elastic component of the network and the loss modulus (G'') is a measure of the viscous component. Tan delta ($\tan \delta = G''/G'$) is a measure of the energy lost due to viscous flow compared to the energy stored due to elastic deformation in a single deformation cycle. A change in tan delta indicates the type of network formed; lower tan delta values represents formation of better three-dimensional network (Sun and Arntfield, 2010). In the beginning of the gelation process, the viscous behavior ($G'' > G'$) dominates the system, whereas the elastic behavior

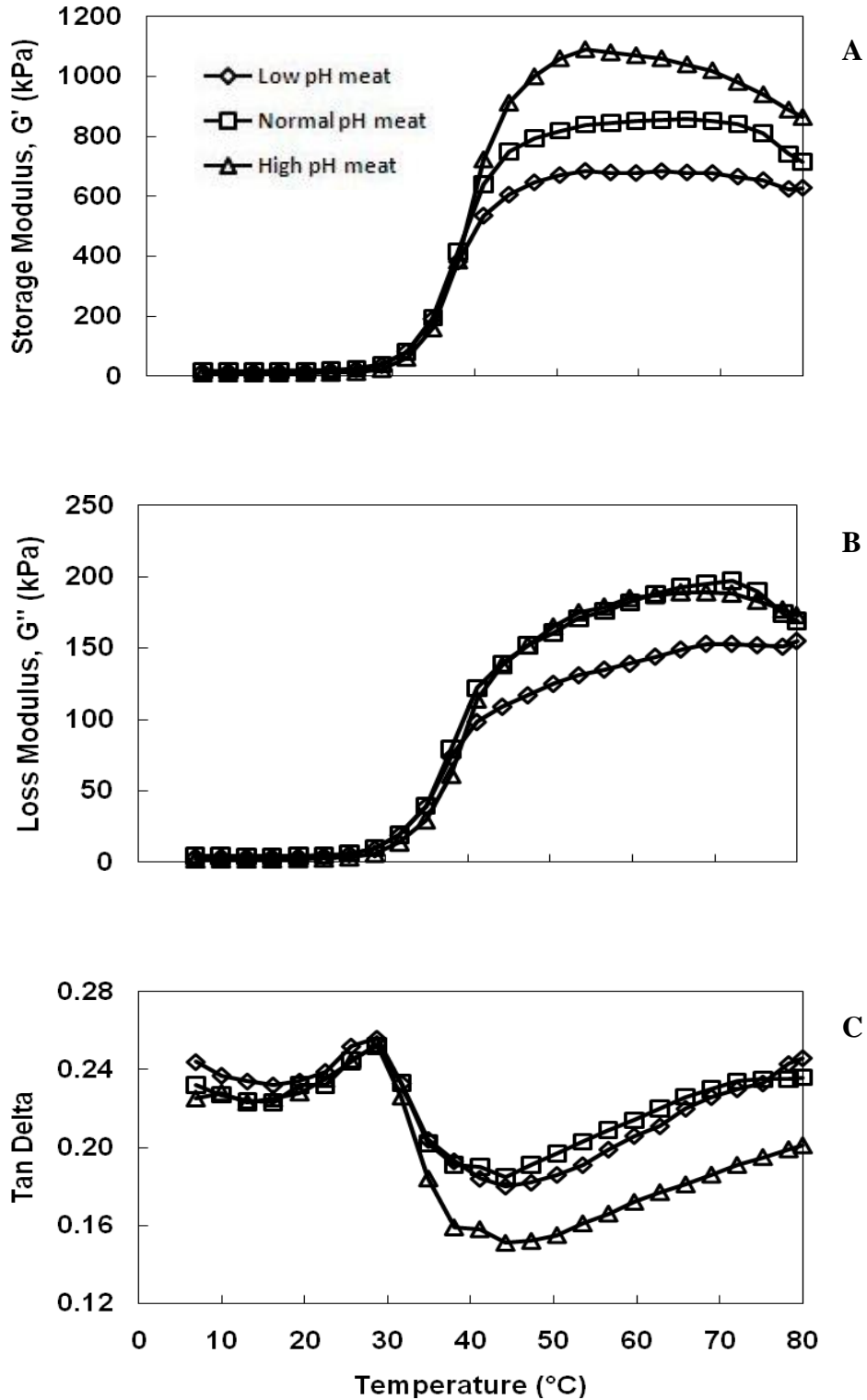


Figure 4.2. Rheograms of low, normal, and high pH meat during heating (6.89°C to 80°C); A: Storage modulus (G'), B: Loss modulus (G''), C: Tan delta ($\tan \delta$).

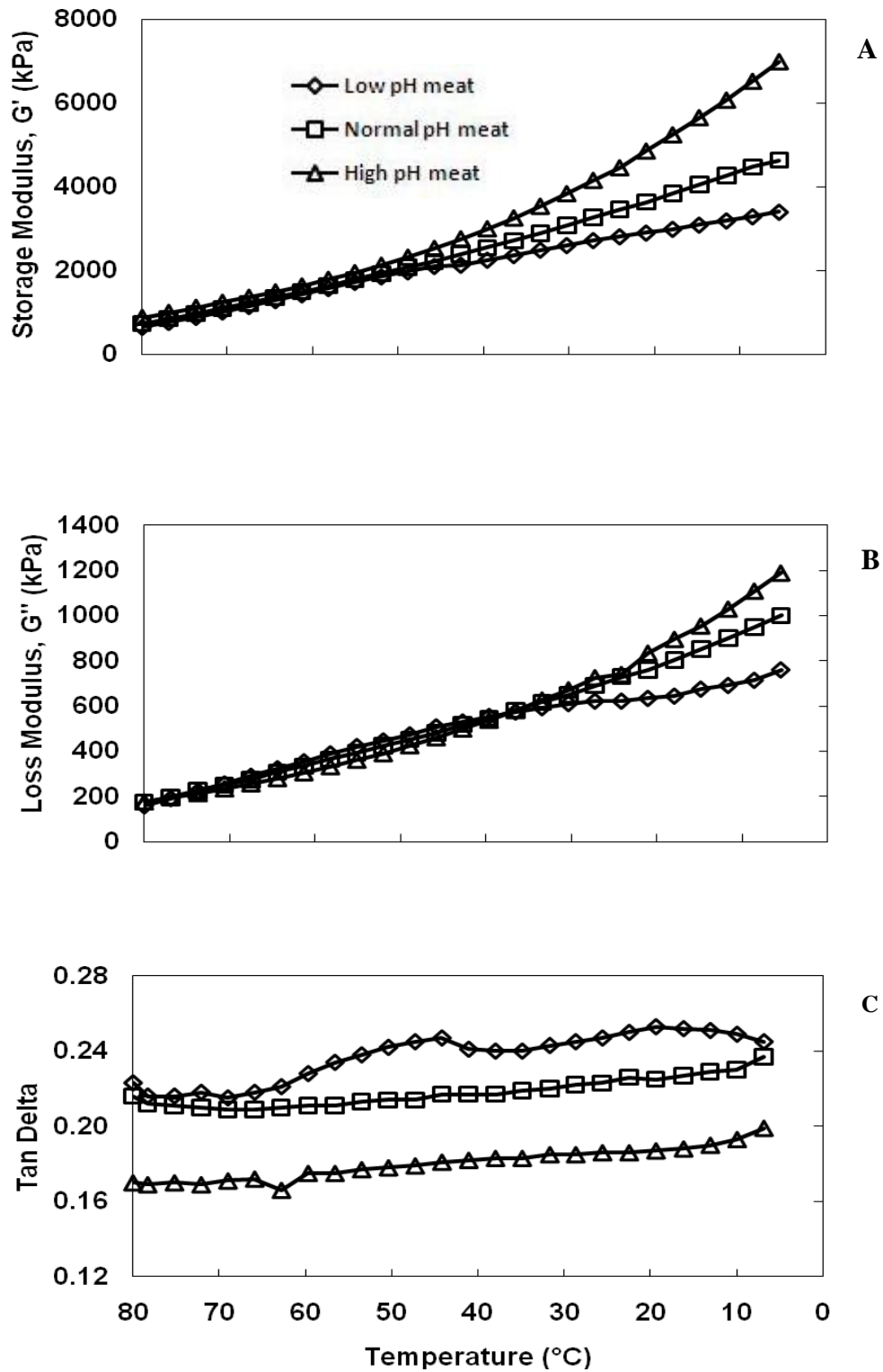


Figure 4.3. Rheograms of low, normal, and high pH meat during cooling (80°C to 6.89°C); A: Storage modulus (G'), B: Loss modulus (G''), C: Tan delta ($\tan \delta$).

dominates the final stages of heating ($G' > G''$) resulting in induction of cross-linking of protein molecules to form a three-dimensional network (Sun and Arntfield, 2010). In the beginning of the heating process at 6.89°C, the G' values of low and normal pH meat were not statistically different (Figure 4.4A). The maximum rate of increase in G' values was in the temperature range of 30°C to 45°C (Figure 4.2A). The maximum G' values for low (676 kPa), normal (793 kPa) and high pH meat (1093 kPa) occurred at approximately 56.6°C (Figure 4.4B). However, there was no statistical difference in the maximum G' values for low and normal pH meat, which further confirmed that these two classes of meat had similar gel strength. In contrast, high pH meat had significantly higher maximum G' values compared to low pH meat, which reveals that high pH meat had formed a stronger intermolecular network and had better gel forming ability. An increase in G' value during heating shows that proteins underwent ordered aggregation and formation of a three-dimensional network with entrapment of water in the matrix (Dileep et al., 2005). The forces responsible for gelation are hydrophobic interactions, disulphide cross bridges and hydrogen bonds (Hamann, 1992). At the end of the heating process at 80°C, the G' values of low, normal, and high pH meat were not statistically different. The loss modulus values of low, normal, and high pH meat also increased during heating, indicating formation of a viscoelastic gel network (Figure 4.2B). During heating, tan delta values of high pH meat were lower than those of low and normal pH meat, which suggests that high pH meat had formed a better three-dimensional network (Figure 4.2C). In muscle proteins,

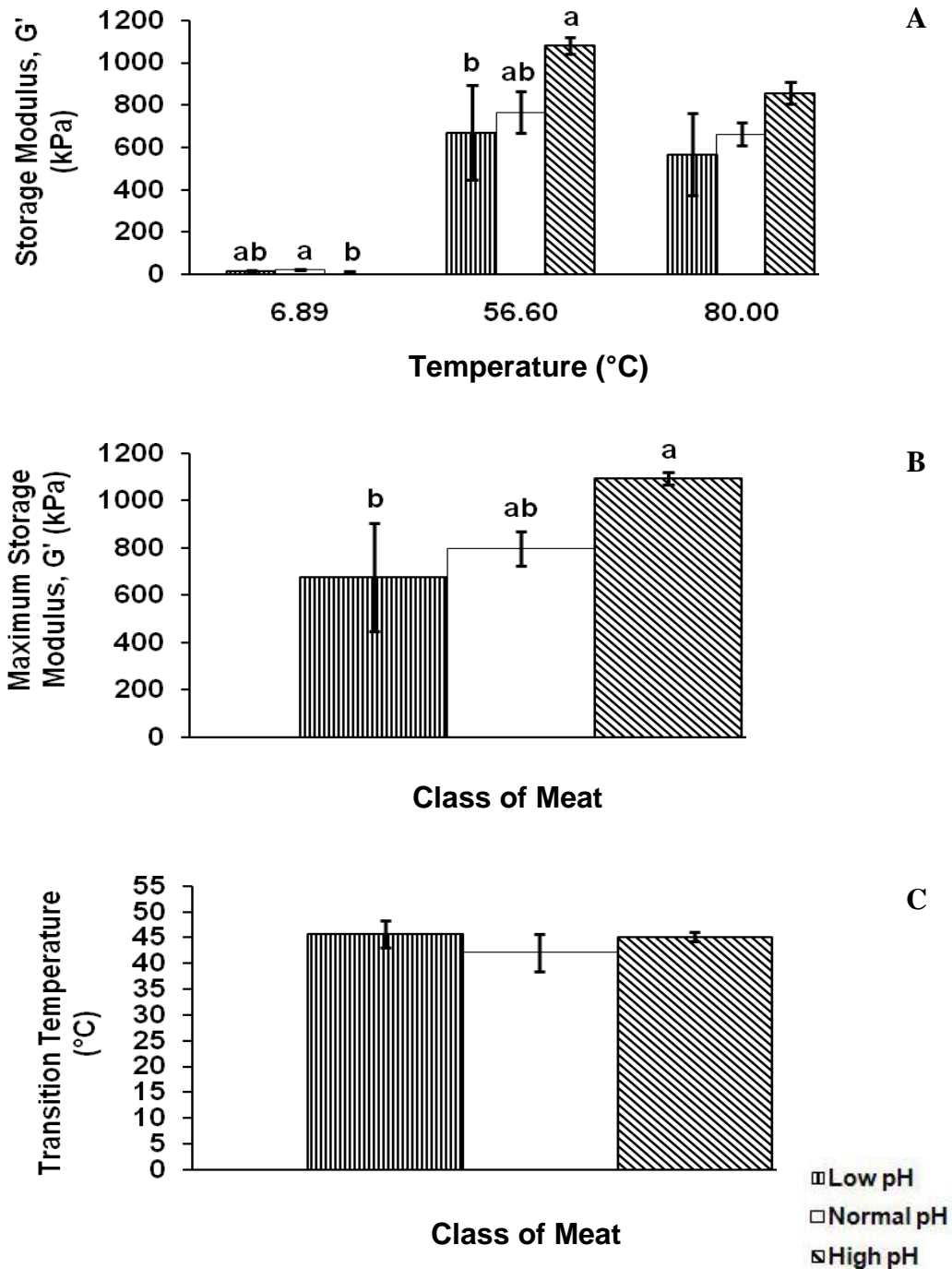


Figure 4.4. A: Storage modulus (G') values at 6.89°C, 56.60°C and 80.00°C of low, normal, and high pH meat, B: Maximum storage modulus (G') values of low, normal, and high pH meat, C: Transition temperature of low, normal, and high pH meat based on $\tan \delta$ ($\tan \delta$) values. Results are presented as means \pm standard deviations ($n=3$). Dissimilar superscripts within each analysis denote significant difference ($P < 0.05$).

tan delta values denote two transitions; first one at 52°C (due to denaturation of myosin) and the second one at 70°C (due to denaturation of collagen) (Westphalen et al., 2005; Brunton et al., 2006). In this study, one major transition for low, normal, and high pH meat was evident at 45.7, 42.1 and 45.2°C, respectively (Figure 4.4C), which was the temperature of myosin denaturation. However, the transition for the three classes of meat was not statistically different, which revealed that the temperature of myosin denaturation was independent of pH₂₄. Omana et al. (2010) found a myosin denaturation temperature of 50.1°C in proteins from chicken dark meat, which indicate that different myosin isoforms in red and white fibers respond differently to denaturing conditions (Van Laack and Lane, 2000). The second transition due to denaturation of collagen was not evident in the present study. This may be due to restraining of collagen fibers during heating which resulted in an increase in denaturation temperature even up to 85°C (Rochdi et al., 1985). This may also be due to the low amount of collagen present in breast meat.

During cooling, G' and G'' values of low, normal, and high pH meat increased steadily, which indicates formation of cross-links and rearrangement of the network structure (Figure 4.3A,B). After cooling, G' and G'' values of high pH meat were higher than that of low and normal pH meat. This increase was due to the formation of hydrogen bonds, which contributed to the stability and elasticity of myosin gel networks (Hamann, 1988). Tan delta values of low, normal, and high pH meat increased slightly and remained constant at a low level (Figure 4.3C). This indicated that a stable gel had formed and the formation of hydrogen

bonds during the cooling phase added strength to both the elastic and viscous components to the network (Sun and Arntfield, 2010). The data suggests that the rigidity of the gel increased with heating and the strengthening of the gel network continued during cooling.

4.4 CONCLUSION

Investigation of the effects of pH₂₄ on functional properties of proteins in frozen turkey breast meat revealed that ultimate pH did not cause major changes to the emulsifying and foaming properties of the extracted sarcoplasmic and myofibrillar proteins. Low and normal pH meat had the same extent of protein denaturation, while low pH meat had lower water holding capacity (WHC) as revealed by increase in cooking loss, which can be explained by factors other than protein denaturation. Gel strength analysis and folding test showed that gel forming ability was better in high pH meat compared to low and normal pH meat. Dynamic viscoelastic behavior (DVB) showed that low, normal, and high pH meat had the same myosin denaturation temperature. Normal and high pH meat had similar textural characteristics. The results from this study indicate that high pH meat is more suitable for further processed products because it has similar or better functional properties than normal pH meat. This study also supported the hypothesis that other biochemical mechanisms, rather than protein denaturation, affects functionality of PSE-like poultry meat.

4.5 REFERENCES

- Allen, C. D., S. M. Russell, and D. L. Fletcher. 1997. The relationship of broiler breast meat color and pH to shelf-life and odor development. *Poult. Sci.* 76:1042-1046.
- Alvarado, C. Z. and A. R. Sams. 2004. Turkey carcass chilling and protein denaturation in the development of pale, soft, and exudative meat. *Poult. Sci.* 83:1039-1046.
- Barbut, S. 1993. Color measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. *Food Res. Int.* 26:39-43.
- Barbut, S. 1995. Importance of fat emulsification and protein matrix characteristics in meat batter stability. *J. Muscle Food* 6:161-177.
- Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. *Br. Poult. Sci.* 38:355-358.
- Brunton, N. P., J. G. Lyng, L. Zhang, and J. C. Jacquier. 2006. The use of dielectric properties and other physical analyses for assessing protein denaturation in beef *Biceps femoris* muscle during cooking from 5 to 85°C. *Meat Sci.* 72:236-244.
- Caine, W. R., J. L. Aalhus, D. R. Best, M. E. R. Dugan, and L. E. Jeremiah. 2003. Relationship of texture profile analysis and Warner-Bratzler shear force with sensory characteristics of beef rib steaks. *Meat Sci.* 64:333-339.
- Chen, T., G. Zhou, X. Xu, G. Zhao, and C. Li. 2010. Phospholipase A₂ and antioxidant enzyme activities in normal and PSE pork. *Meat Sci.* 84:143-146.

- Das, K. P. and J. E. Kinsella. 1990. Stability of food emulsions: Physicochemical role of protein and non-protein emulsifiers. *Adv. Food Nutr.* 34:81-201.
- Dileep, A. O., B. A. Shamasundar, P. K. Binsi, F. Badi, and N. K. Howell. 2005. Effect of ice storage on the physicochemical and dynamic viscoelastic properties of ribbonfish (*Trichiurus spp*) meat. *J. Food Sci.* 70:E537-E545.
- Fraqueza, M. J., A. S. Cardoso, M. C. Ferreira, and A. S. Barreto. 2006. Incidence of *Pectoralis major* turkey muscles with light and dark color in a Portuguese slaughterhouse. *Poult. Sci.* 85:1992-2000.
- Fujii, J., K. Otsu, F. Zorzato, S. de Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science* 253:448-451.
- Gornall, A. G., J. C. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751-766.
- Guardia, M. D., J. Estany, S. Balasch, M. A. Oliver, M. Gispert, and A. Diestre. 2005. Risk assessment of DFD meat due to pre-slaughter conditions in pigs. *Meat Sci.* 70:709-716.
- Hamann, D. D. 1988. Rheology as a means of evaluating muscle functionality of processed foods. *Food Tech.* 42:66-71.
- Hamann, D. D. 1992. Rheological studies of fish proteins. Pages 4-49 in *International Conference and Industrial Exhibition on Food Hydrocolloids*. D. D. Hamann, ed. Japanese Society of Food Hydrocolloids, Tokyo, Japan.

- Huff-Lonergan, E. and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* 71:194-204.
- Hughes, P. S. and E. D. Baxter. 2001. Beer: Quality, Safety and Nutritional Aspects. P. S. Hughes and E. D. Baxter, eds. The Royal Society of Chemistry, UK.
- Kinsella, J. E. 1981. Functional properties of proteins: Possible relationships between structure and function in foams. *Food Chem.* 7:273-288.
- Laemelli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
- Lanier, T. C. 1986. Functional properties of surimi. *Food Tech.* 40:107-114.
- Martinez, O., J. Salmeron, M. D. Guillen, and C. Casas. 2004. Texture profile analysis of meat products treated with commercial liquid smoke flavourings. *Food Control* 15:457-461.
- McKee, S. R. and A. R. Sams. 1998. Rigor mortis development at elevated temperatures induces pale exudative turkey meat characteristics. *Poult. Sci.* 77:169-174.
- Mohan, M., D. Ramachandran, and T. V. Sankar. 2006. Functional properties of Rohu (*Labeo rohita*) proteins during iced storage. *Food Res. Int.* 39:847-854.
- Molette, C., H. Rémignon, and R. Babilé. 2003. Maintaining muscles at a high post-mortem temperature induces PSE-like meat in turkey. *Meat Sci.* 63:525-532.

- Moure, A., H. Dominguez, M. E. Zuniga, C. Soto, and R. Chamy. 2002. Characterization of protein concentrates from pressed cakes of Guevina avellana (*Chilean hazelnut*). *Food Chem.* 78:179-186.
- Nakai, S. and H. W. Modler. 1996. *Food proteins: Properties and characterization*. S. Nakai and H. W. Modler, eds. Wiley-VCH Inc., USA.
- National Chicken Council. 2009. National Chicken Council. http://www.nationalchickencouncil.com/statistics/stat_detail.cfm?id=7. Accessed July 2010.
- Nowsad, A. A. K. M., S. Kanoh, and E. Niwa. 2000. Thermal gelation characteristics of breast and thigh muscles of spent hen and broiler and their surimi. *Meat Sci.* 54:169-175.
- Offer, G. and P. Knight. 1988. The structural basis of water-holding in meat. Pages 173-243 in *Developments in Meat Science*. R. A. Lawrie, ed. Elsevier Applied Science, London, UK.
- Omana, D. A., V. Moayedi, Y. Xu, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Textural properties and color characteristics of recovered proteins. *Poult. Sci.* 89:1056-1064.
- Owen, R. F. 1996. Food Chemistry. Pages 381-382, 909 in *Food Chemistry*. 3rd ed. R. F. Owen, ed. Marcel Dekker, Inc., USA.
- Owens, C. M., E. M. Hirschler, S. R. McKee, R. Martinez-Dawson, and A. R. Sams. 2000. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. *Poult. Sci.* 79:553-558.

- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Pearce, K. N. and J. E. Kinsella. 1978. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J. Agric. Food Chem.* 26:716-723.
- Petracci, M., M. Bianchi, M. Betti, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. *Poult. Sci.* 83:2086-2092.
- Poultry Marketplace. 2010. World future outlook of the chicken meat market. http://www.agr.gc.ca/poultry/prindc2_eng.htm.
- Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color and pH, moisture, water-holding capacity, and emulsification capacity. *Poult. Sci.* 80:676-680.
- Ramachandran, D., M. Mohan, and T. V. Sankar. 2007. Physicochemical characteristics of muscle proteins from barracuda (*Sphyraena jello*) of different weight groups. *Lwt-Food Sci. Tech.* 40:1418-1426.
- Rathgeber, B. M., M. D. Pato, J. A. Boles, and P. J. Shand. 1999. Rapid post-mortem glycolysis and delay chilling of turkey carcasses cause alterations to protein extractability and degradation of breast muscle proteins. *J. Agric. Food Chem.* 47:2529-2536.
- Rochdi, A., M. Bonnet, and J. Kopp. 1985. Denaturation of muscle collagen during heating. Effect of restraint of fibers on shrinkage and degree of denaturation. *Sci. des. Ali.* 5:293-298.

- Santos, C., L. C. Roserio, H. Goncalves, and R. S. Melo. 1994. Incidence of different pork quality categories in a Portuguese slaughterhouse: A survey. *Meat Sci.* 38:279-287.
- Selmane, D., V. Christophe, and D. Gholamreza. 2008. Extraction of proteins from slaughterhouse by-products: Influence of operating conditions on functional properties. *Meat Sci.* 79:640-647.
- Smith, D. P. and J. K. Northcutt. 2009. Pale poultry muscle syndrome. *Poult. Sci.* 88:1493-1496.
- Soares, A. L., E. I. Ida., S. Miyamoto, F. J. Hernández-Blazquez, R. Olivo, J. W. Pinheiro, and M. Shimokomaki. 2003. Phospholipase A₂ activity in poultry PSE, pale, soft, exudative meat. *J. Food Biochem.* 27:309-320.
- Strasburg, G. M. and W. Chiang. 2009. Pale, soft, exudative turkey - The role of ryanodine receptor variation in meat quality. *Poult. Sci.* 88:1497-1505.
- Sun, X. D. and S. D. Arntfield. 2010. Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Res. Int.* 43:509-515.
- Townsend, A. A. and S. Nakai. 1983. Relationships between hydrophobicity and foaming characteristics of food proteins. *J. Food Sci.* 48:588-594.
- Tsaliki, E., U. Kechagia, and G. Doxastakis. 2002. Evaluation of the foaming properties of cottonseed protein isolates. *Food Hydro.* 16:645-652.
- Van Laack, R. L. J. M. and J. L. Lane. 2000. Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. *Poult. Sci.* 79:105-109.

- Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* 79:1057-1061.
- Velleman, S. G. 1998. What's outside the cell and how does it affect avian bone and muscle formation? Pages 71-88. U.S.A.
<http://www.poultryscience.org/pba/1952-2003/1998/1998%20Velleman.pdf>
- Velleman, S. G. 2000. The role of the extracellular matrix in skeletal development. *Poult. Sci.* 79: 985-989.
- Wang, R. R., X. J. Pan, and Z. Q. Peng. 2009. Effects of heat exposure on muscle oxidation and protein functionalities of *pectoralis majors* in broilers. *Poult. Sci.* 88:1078-1084.
- Westphalen, A. D., J. L. Briggs, and S. M. Lonergan. 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. *Meat Sci.* 70:293-299.
- Wild, P. J. and D. C. Clark. 1996. Foam formation and stability. Pages 110-148 in *Methods of Testing Protein Functionality*. P. J. Wild and D. C. Clark, eds. Blackie Academic and Professional, London, England.
- Wilhelm, A. E., M. B. Maganhini, F. J. Hernandez-Blazquez, E. I. Ida, and M. Shimokomaki. 2010. Protease activity and the ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. *Food Chem.* 119:1201-1204.
- Wilkins, L. J., S. N. Brown, A. J. Phillips, and P. D. Warriss. 2000. Variation in the colour of broiler breast fillets in the UK. *Br. Poult. Sci.* 41:308-312.

- Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poult. Sci.* 81:579-584.
- Xiong, Y. L. and C. J. Brekke. 1991. Protein extractability and thermally induced gelation properties of myofibrils isolated from pre- and postrigor chicken muscles. *J. Food. Sci.* 56:210-215.
- Yamamoto, K. and K. Maruyama. 1973. Interaction of troponin I and tropomyosin. *J. Biochem.* 73:1111-1114.
- Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.
- Ziober, I. L., F. G. Paião, D. F. Marchi, L. L. Coutinho, E. Binneck, A. L. Nepomuceno, and M. Shimokomaki. 2010. Heat and chemical stress modulate the expression of the α -RZR gene in broiler chickens. *Genet. Mol. Res.* 9:1258-1266.

CHAPTER 5. APPLICATION OF HIGH PRESSURE PROCESSING TO IMPROVE THE FUNCTIONAL PROPERTIES OF PALE, SOFT, AND EXUDATIVE (PSE)-LIKE TURKEY MEAT⁴

5.1 INTRODUCTION

Global turkey production has been growing steadily worldwide over the past few decades. Traditionally the poultry industry market was primarily on whole birds and cut-up products. More recently, processors have adapted to changing trends as consumers increase their demand for more convenient, ready to eat products (Poultry Marketplace, 2010). Increasing occurrence of a pale, soft, exudative (PSE)-like meat condition in turkeys, similar to the one observed in pigs, has become a major concern in the poultry industry since it affects important meat quality attributes involved in the production of value-added products. In pigs, the combination of rapid *post mortem* pH decline and high carcass temperature causes protein denaturation in the muscle, which leads to reduced protein functionality, such as decreased water holding capacity (WHC) (Santos et al., 1994). The condition in poultry seems to be more related to the extent of post-mortem acidification (i.e. low pH at 24 h) rather than fast *post mortem* pH decline after slaughter (Fraqueza et al., 2006). It has also been shown that in broilers, low *post mortem* muscle pH led to decreased WHC and weaker gel formation (Zhang and Barbut, 2005). Thus, reduced protein functionality in low pH poultry meat may have serious consequences. It leads to an estimated economic loss of more than U.S. \$200 million per year in the turkey industry in further processed

⁴ A version of this chapter was submitted for publication to the journal of *Innovative Food Science and Emerging Technologies*. (Authors: J. T. Y. Chan, D. A. Omana, and M. Betti).

products (Owens et al., 2009). The suitability of PSE meat can be increased for the production of these products by varying processing conditions such as marination with salt or phosphates using tumblers or injectors to increase the juiciness (Barbut, 2009). However, the addition of these ingredients may pose a problem for consumers due to health consciousness and a demand for additive-free products.

High pressure processing (HPP) is currently being used by the meat industry as a post-processing technology to extend shelf life and improve the safety of ready to eat meat products (Jofré et al., 2008). High pressure can affect protein conformation and may lead to protein denaturation, aggregation or gelation, depending on factors, such as the protein system, applied pressure and temperature, and the duration of the pressure treatment (Messens et al., 1997). High pressure processing can also be used as a means to improve the functional properties of muscle proteins (Macfarlane, 1974). An important aspect in meat processing is the solubility of the proteins as it is related to many of their functional properties. Studies reported that low pressure treatment at 150 MPa and 200 MPa increased the protein solubility of sheep myofibrillar proteins (Macfarlane, 1974) and chicken myofibrils (Iwasaki et al., 2006), respectively. Partial replacement of additives such as NaCl and polyphosphates is possible using high pressure processing, since it has a similar effect on myofibrillar proteins as the additives. A recent study conducted by Sikes et al. (2009) reported a large increase in myofibrillar protein solubility and improvement in water retention of cooked products and textural properties of low-salt beef sausage

batters with the application of high pressure. Hence, it has been shown that high pressure processing is effective in improving the functional properties of proteins, while allowing low salt levels to be used in food processing of various meat products.

Previous studies have shown that lower muscle pH is associated with lower water holding capacity, as evident in pale turkey meat (Barbut, 1993; Fraqueza et al., 2006). High pressure processing may induce changes in low pH turkey breast meat proteins to positively influence water holding capacity. We hypothesize that high pressure processing used at low pressure levels (50 - 200 MPa) for short time as a pre-treatment before thermal processing can improve the water retention potential of meat proteins. In fact, pre-treatment using high pressure processing before cooking has been shown to improve gel forming ability in fish surimi (Hsu and Jao, 2007). To our knowledge, no studies have been reported on improving protein functionality of PSE-like turkey breast meat with the application of high pressure processing. Hence, the aim of this work was to study the effects of high pressure processing on turkey breast meat with low and normal pH at 24 h *post mortem* and its relationship with pressure induced changes on protein functionality.

5.2 MATERIALS AND METHODS

5.2.1 Sample selection

Turkeys were slaughtered at 106 d of age and the average flock weight was 11.1 kg. A total number of 35 skinless, boneless breast fillets from Hybrid Tom turkeys were initially selected from a local processing plant (Lilydale Inc.,

Edmonton, Alberta, Canada) at 24 h *post mortem*. They consisted of 26 pale and 9 normal fillets. Selection was made using color measurement based on lightness (L^*) values as reported by Zhang and Barbut (2005). The samples were labeled and placed individually in plastic bags, packed on ice and transported to the laboratory. L^* values and ultimate pH at 24 h *post mortem* (pH_{24}) were measured again on all 35 breast samples in the laboratory. Eight breast samples were further selected within each class (pale and normal), according to their pH_{24} . In this experiment, two classes of meat with different pH were selected because ultimate pH has been indicated as a valid tool to differentiate protein functionality. In summary, the color and pH characteristics are reported in Table 5.1. The average L^* and pH values of the samples were within the following range: pale ($L^* > 52$, $pH \leq 5.7$) and normal ($46 < L^* < 52$, $pH \geq 6.0$) and referred to as low and normal pH meat, respectively. Each fillet was labeled according to the class and was minced individually in a Kitchen-Aid food processor (Model KFP 7500B, KitchenAid, St. Joseph, MI, USA) for 2 min. The food processor was pre-chilled and operated in a cold room (4°C) to prevent the temperature of the samples exceeding 10°C throughout the mixing process. The samples within each class were then mixed homogeneously to obtain two batters of low and normal pH meat. The average pH values of low and normal pH meat batters were 5.75 ± 0.06 and 6.01 ± 0.03 , respectively. All the analyses were carried out in frozen meat samples that were vacuum packaged in polyethylene bags and stored at -30°C for 3 weeks and thawed overnight at 4°C .

Table 5.1. Physical properties of low and normal pH meat¹

Measurement	Class of Meat	
	Low pH meat	Normal pH meat
L* (24 h)	52.1 ± 0.9 ^a	46.4 ± 0.8 ^b
a* (24 h)	2.9 ± 0.3 ^b	5.3 ± 0.8 ^a
b* (24 h)	0.84 ± 0.90 ^a	-0.22 ± 0.77 ^b
pH ₂₄	5.68 ± 0.09 ^b	6.08 ± 0.10 ^a

¹Results are presented as means ± standard deviations (n = 8).

Means within each row with no common superscript differ significantly ($P < 0.05$). L* = lightness; a* = redness; b* = yellowness; pH₂₄ = ultimate pH at 24 h *post mortem*.

5.2.2 Color measurements

The methodology for color measurement is described in Chapter 3 (refer to section 3.2.2).

5.2.3 pH measurements

The methodology for pH measurement is described in Chapter 3 (refer to section 3.2.3).

5.2.4 Meat batter formulation for high pressure processing

The formulation of batters used in this study was chosen based on a preliminary study. Batters were prepared with NaCl (0.5% w/w) and varying concentrations of water (10, 20 and 30%) and subjected to high pressure at 100 and 200 MPa. Results showed that high pressure at 100 MPa increased protein solubility and improved water holding capacity of batters with 10% water compared to all other batters with no pressure treatment (control) and pressure treatment at 100 and 200 MPa. Thus, the optimal batter formulation for this study

was determined to contain 10% water. Raw meat batters were prepared by comminuting minced turkey meat (89.5%) with water (10%) and NaCl (0.5%) using a motor and pestle. During preparation, the batter was maintained at a temperature of less than 10°C. The batters were then filled into cryovials (12.0 mm-diameter and 24.0 mm-height) of 2 mL capacity for high pressure treatments.

5.2.5 High pressure processing

Pressure treatments were performed using a U111 high pressure multivessel apparatus (UNIPRESS Equipment Division, Warsaw, Poland), which has a maximum pressure limit of 800 MPa and is capable of operating with temperature between 0°C and 120°C. The apparatus has four pressure chambers to hold samples, each with a maximum sample envelope dimension of 12.4 mm-diameter and 60.0 mm-height. The pressure medium used in the sample chambers was propylene glycol. The apparatus is thermostated by a heat exchanger connected to an external circulator. The temperature was maintained by a thermostating circulator bath (Lauda Proline RP 855 Low Temperature Thermostat, GMBH & Co. Lauda-Konigshofen, Germany). The apparatus has one high pressure transducer between the intensifier and the vessels, which monitored the pressure profile during treatment cycles. The batters were subjected to 50, 100, 150 and 200 MPa at 4°C and were held for 5 min. The time required to reach pressure was 20, 30, 38, and 43 secs for 50, 100, 150, and 200 MPa, respectively. All samples were then kept at 4°C for analyses.

5.2.6 Protein solubility

Sarcoplasmic and total protein solubility was determined according to the method as described by Van Laack et al. (2000) with modifications. For sarcoplasmic protein solubility, 2 g of meat was homogenized with 40 mL 0.03 M phosphate buffer (pH 7.4). Homogenates were centrifuged using Avanti J-E refrigerated centrifuge (Beckman Coulter, Inc., Palo Alto, CA, USA) at 15,300 x g for 15 min at 4°C and filtered through Whatman No. 1 filter paper. The protein concentration of the filtrate (protein solution) was assessed using the Biuret procedure (Gornall et al., 1949) with bovine serum albumin (BSA) as standard. For total protein solubility, 1 g of meat was homogenized with 40 mL 0.05 M phosphate buffer containing 0.55 M KI (pH 7.4). Homogenates were centrifuged, filtered and analyzed for protein concentration as described for the sarcoplasmic protein solubility.

5.2.7 Protein surface hydrophobicity

The methodology for surface hydrophobicity of sarcoplasmic and myofibrillar proteins is described in Chapter 3 (refer to section 3.2.7).

5.2.8 Reactive (free) and total sulfhydryl content

The methodology for reactive (R-SH) and total (T-SH) sulfhydryl content is described in Chapter 3 (refer to section 3.2.8).

5.2.9 Cooking of samples

The samples were heated at 95°C in a water bath until the internal temperature of the samples reached 75°C, at which the samples are considered cooked. The temperature was checked using thermocouples inserted in the center

of the samples. After cooking, the samples were cooled at room temperature for 15 min and stored at 4°C overnight for expressible moisture (cooked samples) and texture profile analyses.

5.2.10 Expressible moisture

The methodology for water holding capacity (WHC) of raw and cooked samples as measured by expressible moisture (EM) is described in Chapter 3 (refer to section 3.2.4).

5.2.11 Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

The methodology for SDS-PAGE is described in Chapter 4 (refer to section 4.2.7)

5.2.12 Texture profile analysis

The methodology for texture profile analysis (TPA) is described in Chapter 4 (refer to section 4.2.8).

5.2.13 Dynamic viscoelastic behavior

The methodology for dynamic viscoelastic behavior (DVB) is described in Chapter 4 (refer to section 4.2.10).

5.2.14 Statistical Analysis

Each assay was carried out in 4 replications per meat type. Reported results represent an average of each experimental assay. The data were analyzed as a 2 x 5 factorial ANOVA using the Mixed procedure of SAS (SAS version 9.0, SAS Institute, Cary, NC, USA, 2006). The model tested the main effects for meat pH group (low and normal) and treatments (control, 50 MPa, 100 MPa, 150 MPa

and 200 MPa) as well as the interaction term using residual errors. Differences between group means were determined using HSD Tukey differences and were reported as significant at the $P < 0.05$ level.

5.3 RESULTS AND DISCUSSION

5.3.1 Protein solubility

Solubility of proteins is an important aspect in meat processing because it is related to many functional properties. Protein solubility is a good indicator of protein denaturation (Van Laack et al., 2000). In the present study, the effect of high pressure on total protein solubility was found to be dependent on the classes of meat and the intensity of the treatments as indicated by the significant interaction ($P < 0.05$) reported in Figure 5.1A. Total protein solubility of low and normal pH meat for the control samples was 101.9 mg/g and 103.4 mg/g, respectively, and was not statistically different, which is consistent with the results in Chapter 3. In general, application of high pressure processing caused a significant increase in total protein solubility in both low and normal pH meat compared to that of control (unpressurized) samples. Iwasaki et al. (2006) found an increase in protein solubility of chicken myofibrils that were subjected to pressure treatment at 200 MPa compared to control samples. It was hypothesized that pressure treatment caused swelling of myofibrils and led to disruption and dispersion into short filaments which increased solvent accessibility to the smaller modified structures, leading to increased solubilisation (Macfarlane, 1974; Sikes et al., 2009) A significant ($P < 0.05$) and large increase in total protein solubility

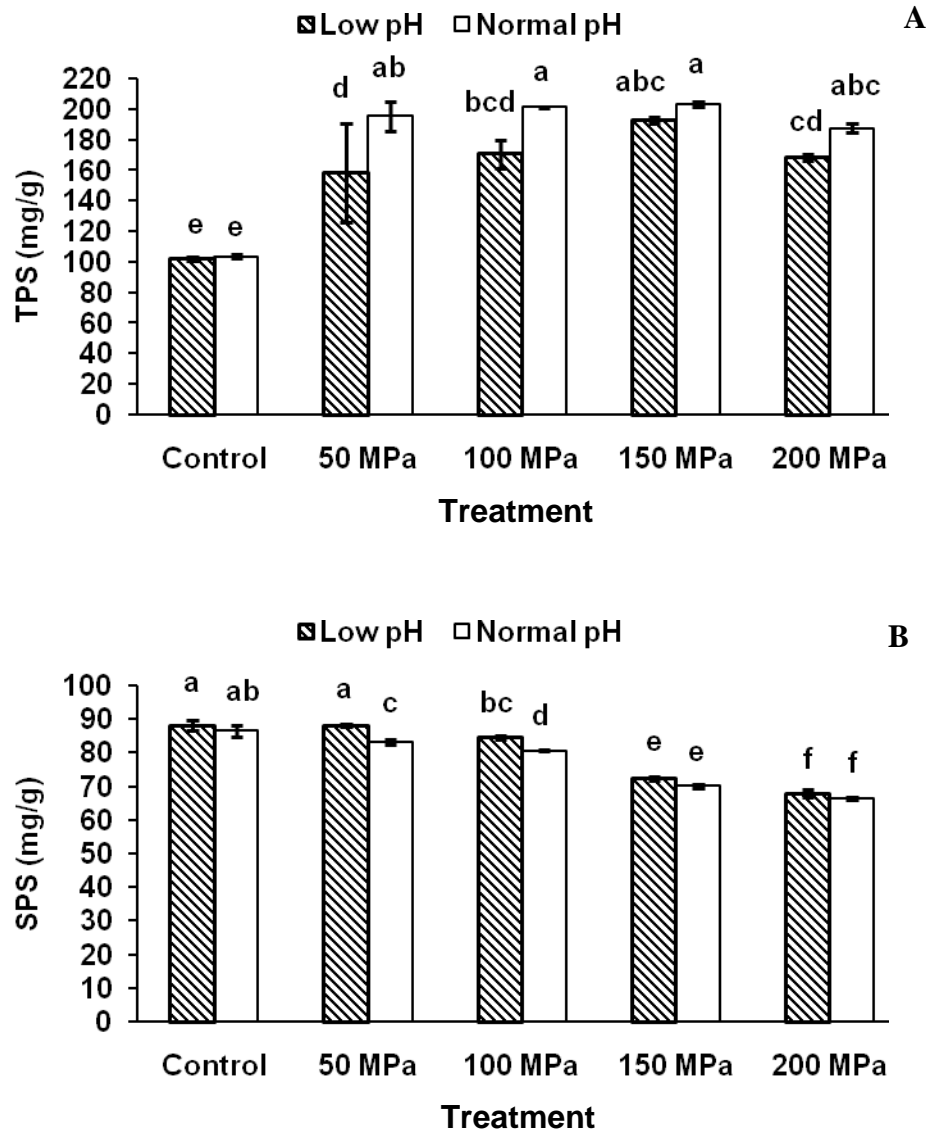


Figure 5.1. Effect of high pressure processing on total and sarcoplasmic protein solubility in low and normal pH meat. A: Total protein solubility (TPS). B: Sarcoplasmic protein solubility (SPS). Dissimilar superscripts denote significant difference ($P < 0.05$). Results are presented as means \pm standard deviations ($n = 4$).

at 50 and 100 MPa in both low and normal pH meat compared to control samples was observed. However, this percentage increase in protein solubility was greater in normal pH meat compared to low pH meat at both pressure treatments (47.0 and 48.6% vs. 35.6 and 40.3%, respectively). This may be the direct consequence of the different pH values in the two classes of meat. Myofibrillar proteins in normal pH meat are further away from the isoelectric point (pI), which is usually at pH 5.3 (Offer and Knight, 1988) than low pH meat, and thus proteins pack more loosely together due to electrostatic repulsion. Therefore, the application of pressure to this type of meat is more effective in promoting depolymerisation of myofibrillar proteins (Cheftel and Culioli, 1997). Conversely, in low pH meat, electrostatic attraction of myofibrillar proteins is high due to the close proximity to the pI, and thus more energy is required to depolymerise the proteins.

In the case of sarcoplasmic protein solubility, a significant interaction ($P < 0.05$) between classes of meat and pressure treatments was observed (Figure 5.1B). Sarcoplasmic protein solubility of low and normal pH meat for the control samples was 88.1 mg/g and 86.5 mg/g, respectively, and was not statistically different. However, sarcoplasmic protein solubility decreased with increasing pressures in both low and normal pH meat. Pressurisation at 200 MPa resulted in the maximum reduction in sarcoplasmic protein solubility for both classes of meat. Marcos et al. (2010) also found a decrease in sarcoplasmic protein solubility of beef muscles that were pressurised at 200 MPa at 10, 20 and 30°C. The decreased protein solubility may be due to cross linking of sarcoplasmic proteins induced by high pressure processing, which was more apparent at processing at

200 MPa as revealed by reduction in reactive sulfhydryl groups (Figure 5.3A). For instance, sarcoplasmic proteins appeared to be covalently linked with a highly compact structure during high pressure processing of fish meat (Ohshima et al., 1993).

5.3.2 Protein surface hydrophobicity

Protein surface hydrophobicity relates to the extent of distribution of hydrophobic residues on the protein surface. The effect of high pressure on myofibrillar protein surface hydrophobicity was found to be dependent on the classes of meat and the pressure treatments as indicated by the significant interaction ($P < 0.05$) reported in Figure 5.2A. There was no significant difference in myofibrillar protein surface hydrophobicity of low (352 Ho) and normal (331 Ho) pH meat for the control samples which was similar to the findings in Chapter 3. In low pH meat, pressure at 50 MPa caused the greatest increase in surface hydrophobicity (450 Ho), however it was not statistically different than that at 100 and 200 MPa. Chapleau and de Lamballerie-Anton (2003) have shown that pressures up to 450 MPa induce a threefold increase in surface hydrophobicity of bovine myofibrillar proteins. Ikeuchi et al. (1992) also observed an increase in surface hydrophobicity of rabbit actomyosin that was pressurized at 150 MPa for 5 min. Hummer et al. (1998) and Tanaka et al. (2000) reported that pressure causes conformational fluctuations of amino acid side chains in proteins. This creates pathways for water to enter into the interior of the protein, which fills the cavities, and thus, causes the opening of the protein structure. Hence, this may

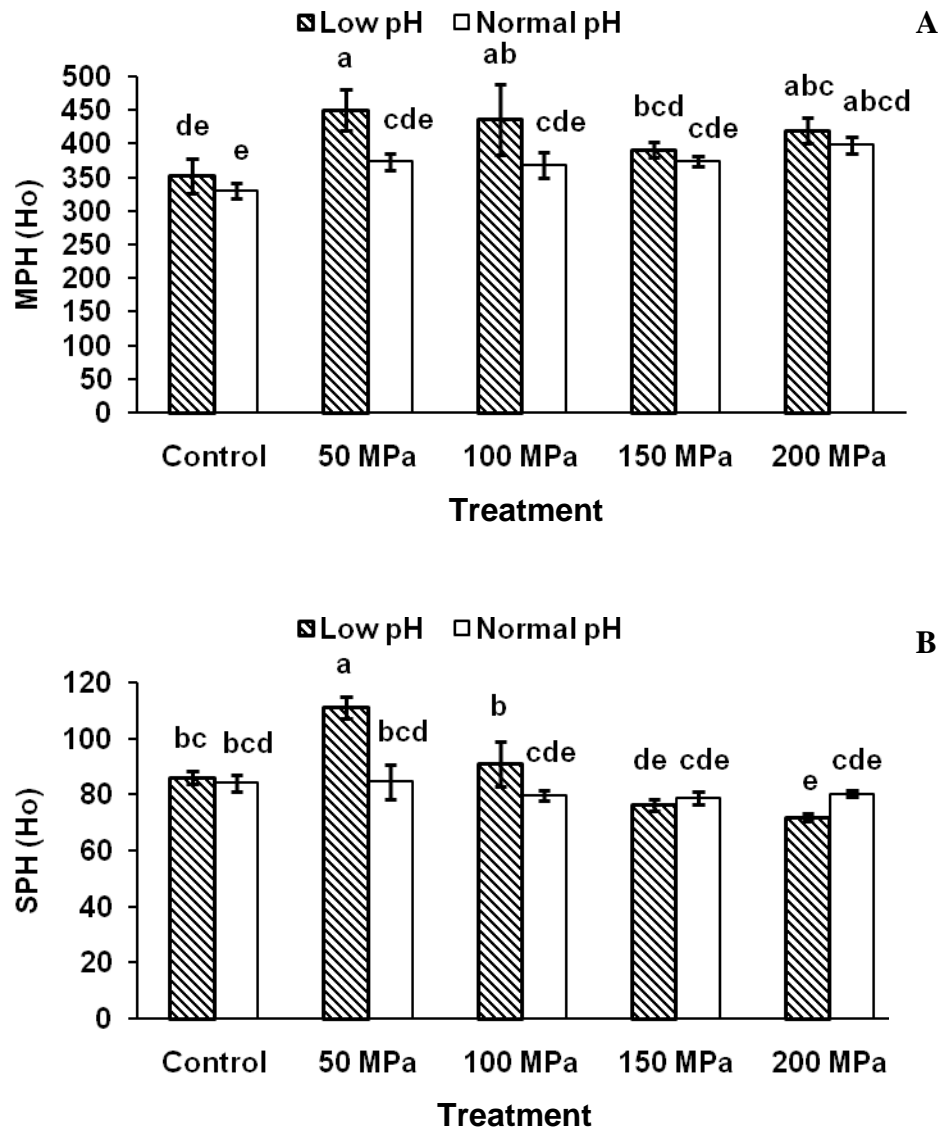


Figure 5.2. Effect of high pressure processing on myofibrillar and sarcoplasmic protein surface hydrophobicity in low and normal pH meat. A: Myofibrillar protein surface hydrophobicity (MPH). Dissimilar superscripts denote significant difference ($P < 0.05$). B: Sarcoplasmic protein surface hydrophobicity (SPH). Dissimilar superscripts denote significant difference ($P < 0.0001$). Results are presented as means \pm standard deviations ($n = 4$).

expose hydrophobic groups on the protein surface leading to higher hydrophobicity.

In the case of normal pH meat, there was no statistical difference in myofibrillar protein surface hydrophobicity of pressure treated samples (Figure 5.2A). This indicated that myofibrillar proteins in normal pH meat were more stable under high pressure compared to that of low pH meat. However, at 50 and 100 MPa, myofibrillar protein surface hydrophobicity was higher in low pH meat compared to that of normal pH meat which indicates more protein unfolding. Hence, the effect of pressure seems to be more evident in low pH meat affecting the structural changes in proteins. Although the mechanism is not immediately clear, some hypotheses are worth considering. It can be speculated that proteins have increased conformational fluctuations in the amino acid side chains in low pH meat during high pressure processing providing more pathways for water to diffuse into the protein interior (Tanaka et al., 2000). As a consequence, this would cause an increased swelling of proteins, which leads to more protein unfolding. Pressure may also alter the structure of the protecting layer of water surrounding the protein surface more effectively in low pH meat inducing conformational changes in proteins (Hayakawa et al., 1996). NMR studies in meat seem to support this hypothesis, where the researchers investigated water compartmentalization and distribution in muscle tissue (Bertram et al., 2007). Earlier study revealed that relaxation times were dependent on pH decrease in *post mortem* muscle (Bertram et al., 2003). They found broad distributions of longer relaxation times in low pH meat than in normal pH meat, which suggests

changes in the water layers surrounding the proteins. Furthermore, in low pH meat, an increase in myofibrillar protein surface hydrophobicity may also be the reason for decreased total protein solubility at 50 and 100 MPa.

Sarcoplasmic protein surface hydrophobicity was affected by the significant interaction ($P < 0.0001$) between muscle pH and pressure treatments (Figure 5.2B). Sarcoplasmic protein surface hydrophobicity of low and normal pH meat for the control samples was 86.1 Ho and 84.1 Ho, respectively, and was not statistically different. Similar findings were also reported in Chapter 3. In low pH meat, pressure applied at 50 MPa caused unfolding of proteins as indicated by significantly higher ($P < 0.0001$) protein surface hydrophobicity compared to all other samples. However, as pressure was increased, surface hydrophobicity decreased, which may reveal that proteins tend to form a more compacted structure resulting in less hydrophobic groups exposed on the surface of proteins. In normal pH meat, no statistical difference in surface hydrophobicity of all samples was found (Figure 5.2B), which showed that sarcoplasmic proteins in normal pH meat were more stable under high pressure compared to that of low pH meat. At 50 and 100MPa, sarcoplasmic protein surface hydrophobicity was higher in low pH meat than in normal pH meat. As explained previously, low pH meat may be more sensitive to pressure, which causes changes in the protecting water layer as evidenced by NMR studies. In low pH meat, the increase in sarcoplasmic protein hydrophobicity may not have affected protein solubility of sarcoplasmic proteins because these types of proteins contain mainly hydrophilic groups which help in solubilisation.

5.3.3 Reactive (free) and total sulfhydryl content

Sulfhydryl groups are one of the most reactive functional groups in proteins. A significant interaction ($P < 0.0001$) was found between muscle pH and pressure treatments on reactive sulfhydryl groups (Figure 5.3A). There was no significant difference in reactive sulfhydryl groups of low (39.9 $\mu\text{mol/g}$ protein) and normal (38.6 $\mu\text{mol/g}$ protein) pH meat for the control samples. This was consistent with the findings in Chapter 3. In low pH meat, pressure at 50 MPa caused the greatest increase in reactive sulfhydryl groups (43.7 $\mu\text{mol/g}$). This may be explained by the greater extent of protein unfolding as shown by high surface hydrophobicity values at 50 MPa (Figure 5.2A,B), and thus, a greater amount of reactive sulfhydryl groups were also exposed on the protein surface at this pressure level. In normal pH meat, pressure at 50 MPa also caused the greatest increase in reactive sulfhydryl groups (44.4 $\mu\text{mol/g}$), although not statistically different. Further increase in pressure to 200 MPa resulted in reduction of exposed reactive sulfhydryl groups.

There was no significant interaction in total sulfhydryl groups between classes of meat and pressure treatments. Total sulfhydryl groups were found to be dependent on pressure effects only ($P < 0.0001$) and are shown in Figure 5.3B. Control samples had the highest amount of total sulfhydryl groups (80.2 $\mu\text{mol/g}$). The total sulfhydryl groups slightly decreased at 50 MPa and remained constant to 200 MPa. Ko et al. (2003) have also found a decrease in total sulfhydryl content in tilapia myosins after pressurization at 50 MPa revealing the formation of disulfide bonds.

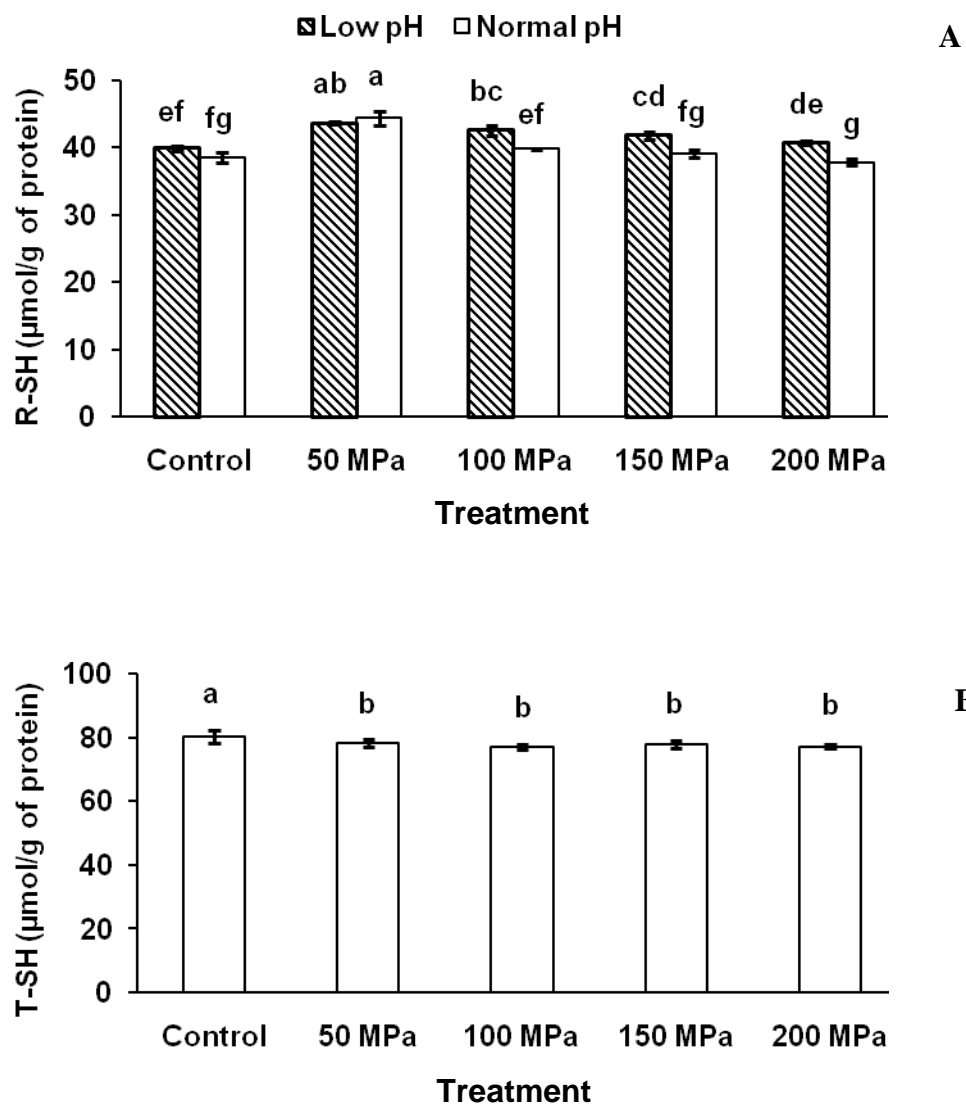


Figure 5.3. Effect of high pressure processing on reactive and total sulfhydryl content. A: Reactive sulfhydryl content (R-SH) in low and normal pH meat. B: Total sulfhydryl content (T-SH) at different pressure treatments. Dissimilar superscripts denote significant difference ($P < 0.0001$). Results are presented as means \pm standard deviations ($n = 4$).

5.3.4 pH

Pressure treatment of meat and meat products is known to produce a small increase in pH (Angsupanich and Ledward, 1998), which may result from a decrease in acidic groups due to conformational changes of proteins associated with denaturation (Poulter et al., 1985). In the present study, the mean pH of low pH meat control samples was 5.51 and was significantly lower ($P < 0.0001$) than that of normal pH meat control samples (5.65) (Table 5.2). Unexpectedly and in contrast to Angsupanich and Ledward (1998), in low pH meat, pressure at 50 and 100 MPa caused a decrease in pH. This may be related to more exposure of acidic groups on the protein surface due to protein unfolding as revealed by surface hydrophobicity data, and hence pH decreases. On the other hand, pressure at 150 and 200 MPa caused an increase in pH. In normal pH meat, pressure at 50 MPa caused an increase in pH, with further increase in pH at 100 MPa, after which it remained stable at 150 and 200 MPa.

Table 5.2. Effect of high pressure processing on pH of low and normal pH meat¹

Class of Meat	Treatment	pH
Low pH meat	Control	5.51 ± 0.01 ^g
	50 MPa	5.35 ± 0.01 ^h
	100 MPa	5.30 ± 0.01 ⁱ
	150 MPa	5.56 ± 0.01 ^f
	200 MPa	5.55 ± 0.01 ^f
Normal pH meat	Control	5.65 ± 0.01 ^e
	50 MPa	5.67 ± 0.01 ^d
	100 MPa	5.83 ± 0.01 ^a
	150 MPa	5.78 ± 0.01 ^c
	200 MPa	5.81 ± 0.01 ^b

¹Results are presented as means ± standard deviations (n = 4). Means within column with no common superscript differ significantly ($P < 0.0001$).

5.3.5 Expressible moisture

Water holding capacity (WHC) is an important property which determines meat quality and is often used to evaluate PSE meat (Woelfel et al., 2002). Expressible moisture, a good indicator of water holding capacity, is the percentage of total water in the meat that can be expressed by applied force. In this study, expressible moisture was conducted on uncooked and cooked samples.

There was a significant interaction ($P < 0.0001$) between the classes of meat and pressure treatments on expressible moisture of uncooked samples (Figure 5.4A). The expressible moisture of low pH meat control (unpressurized) samples was 25.2% and was significantly higher ($P < 0.0001$) than that of normal pH meat (16.9%), which revealed that it had lower WHC compared to normal pH meat. This was consistent with previous work (Barbut, 1993; Fraqueza et al., 2006), which demonstrated that lower muscle pH is associated with lower WHC. In low pH meat, the expressible moisture was decreased at 50 and 100 MPa, with the lowest level occurring at 100 MPa (18.7%), which indicated an increase in WHC at these pressure levels. It was also found that in uncooked low pH meat, pressure at 100 MPa was the best treatment to improve WHC, close to the level as that of normal pH meat control samples. However, at 150 MPa, the expressible moisture was increased to 24.8%, with subsequent decrease at 200 MPa (19.4%). Pressurization at 150 MPa caused the greatest decrease in WHC in low pH meat and was at the same level as control. This may be related to poor gel formation with less water entrapment due to less hydrophobic interactions, as indicated by lower protein surface hydrophobicity at 150 MPa. Lakshmanan et al. (2007) also

observed the highest decrease in WHC in salmon with pressurization at 150 MPa. For normal pH meat, the expressible moisture of the samples at 50 and 100 MPa were not statistically different than that of control samples, although the lowest level occurred at 100 MPa (14.2%). At 150 MPa, the expressible moisture was increased to 21.8%, with subsequent decrease at 200 MPa (17.6%).

A significant interaction ($P < 0.0001$) was found between the classes of meat and pressure treatments on the expressible moisture of cooked samples (Figure 5.4B). The expressible moisture of low pH meat control samples was 18.3% and was significantly higher ($P < 0.0001$) than that of normal pH meat (15.2%), which also revealed that it had lower WHC compared to normal pH meat. Sikes et al. (2009) have found that treatment at 200 MPa resulted in significant improvement in WHC as revealed by lower cook loss in beef batters containing 0.6% salt compared to control samples. In the present study, pressure at 50 MPa seems to be the best treatment to improve WHC of cooked low pH meat, close to the level as that of normal pH meat control samples. However, pressures above 50 MPa caused a slight increase in expressible moisture. In normal pH meat, treatment at all pressure levels did not cause further decrease in expressible moisture as compared to control samples.

5.3.6 Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE profile of total, sarcoplasmic, and myofibrillar proteins extracted from low and normal pH meat is shown in Figure 5.5. The major proteins which contribute to most of the functional properties of myofibrillar

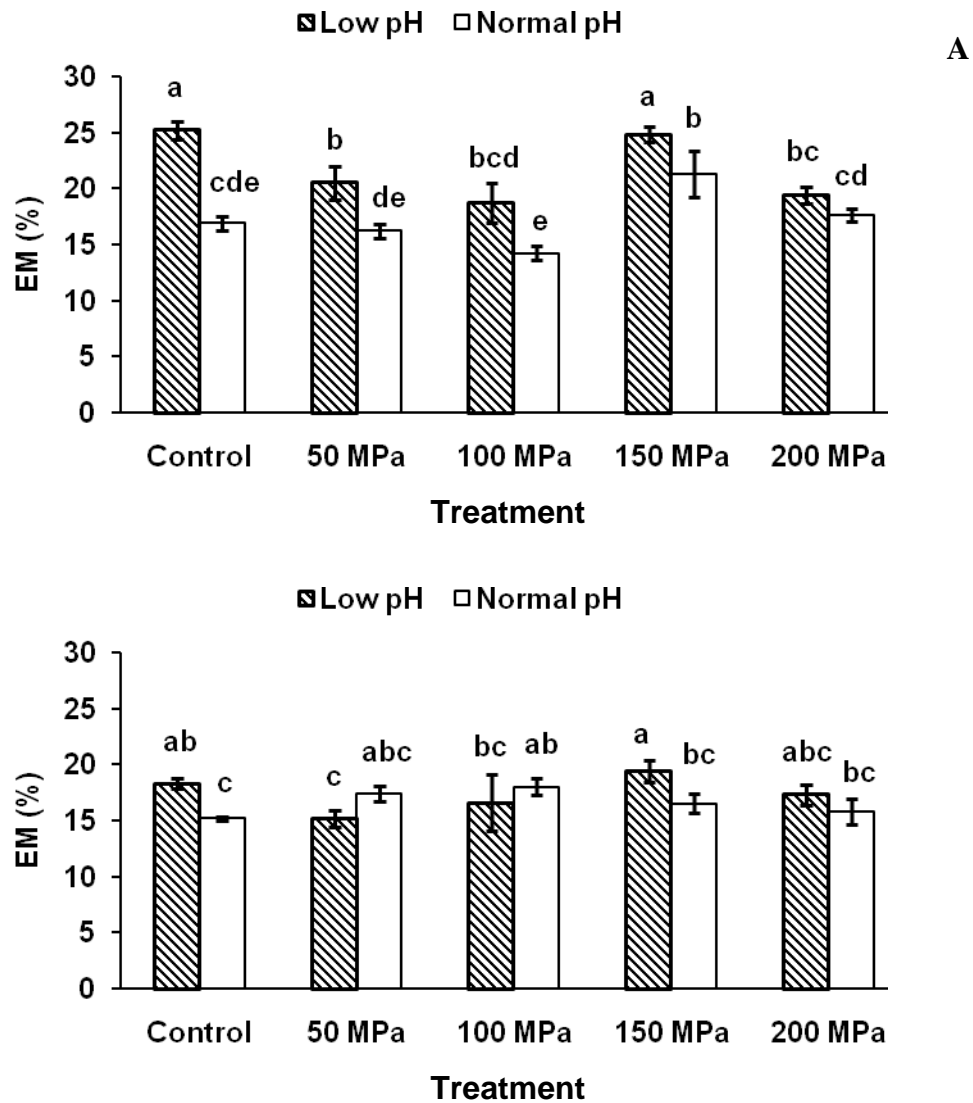


Figure 5.4. Effect of high pressure processing on expressible moisture in low and normal pH meat. A: Expressible moisture (EM) in uncooked samples. B: Expressible moisture in cooked samples. Dissimilar superscripts denote significant difference ($P < 0.0001$). Results are presented as means \pm standard deviations ($n = 4$).

proteins are myosin and actin. In the control samples, the electrophoretic band patterns of proteins from low and normal pH meat in total (lanes 2 and 3 in Figure 5.5A), sarcoplasmic (lanes 2 and 3 in Figure 5.5B), and myofibrillar (lanes 2 and 3 in Figure 5.5C) fractions were similar. In the SDS-PAGE profile of total proteins (Figure 5.5A), the band intensities were less at higher pressure levels, which was more apparent in low pH meat than in normal pH meat. The most significant change that occurred in low pH meat was at 200 MPa (lane 7), where the concentration of myosin heavy chain decreased compared to other samples. This may be due to aggregation as indicated by reactive sulfhydryl group data (Figure 5.3A). This aggregation is mainly due to intermolecular disulfide bond formation at higher pressure levels (Angsupanich et al., 1999). In the SDS-PAGE profile of sarcoplasmic proteins (Figure 5.5B), the band intensities were less above 50 and 100 MPa in low (lanes 5, 6, and 7) and normal pH meat (lanes 10 and 11), respectively, specifically the band at approximately 100 kDa. The decreased band intensities may be caused by protein denaturation leading to protein degradation or insolubilization of sarcoplasmic proteins (Marcos et al., 2010). Under high pressure, certain sarcoplasmic proteins can become covalently linked together rather than be degraded into lower molecular weight components and thus, they become resistant to extraction with SDS, hence the intensity of bands were reduced (Ohshima et al., 1993). In the SDS-PAGE profile of myofibrillar proteins (Figure 5.5C), the decreased concentration of myosin heavy chain was also evident in low pH meat at 200 MPa (lane 7). In low (lanes 6 and 7) and normal (lanes 10 and 11) pH meat at 150 and 200 MPa, the band intensities

corresponding to α -actinin (95 kDa), actin (43 kDa), and tropomyosin (36 kDa) were less intense compared to other samples, which revealed insolubilisation of these proteins under pressure treatment. Chapleau and de Lamballerie-Anton (2003) have found the disappearance of α -actinin band in bovine myofibrillar protein fraction after pressurization above 300 MPa.

5.3.7 Texture profile analysis

Textural characteristics are important in the production of further processed meat products because they affect consumer acceptability. Moisture content of low and normal pH meat used for texture profile analysis was approximately 77% and was not statistically different in control and pressure treated samples for the two classes of meat. Texture profile analysis of low and normal pH meat for different treatment is presented in Table 5.3. The textural characteristics of control samples of low and normal pH meat were not statistically different. Sikes et al. (2009) observed an increase in hardness of beef sausage batters with 0.5% salt that were treated at 200 MPa compared to control samples. In the present study, no difference was found in hardness between samples treated at higher pressure levels and control. This may be due to different types of meat used in the batter preparation. Springiness is the ability of a sample to recover its original form after deformation and chewiness is the work needed to chew a sample to a steady state of swallowing (Martinez et al., 2004). Springiness and chewiness gives an indication of juiciness and tenderness, respectively. In low pH meat, pressure at 200 MPa caused an increase in springiness compared to that of control samples, while in normal pH meat, the springiness in all pressure

treated samples were not different from the control samples. In low and normal pH meat, pressurization did not cause further increase in chewiness, while pressure above 100 MPa resulted in greater cohesiveness in samples. Pressure at 200 MPa caused the largest increase in resilience in the two classes of meat. A complete sensory panel evaluation is needed to further understand consumer acceptance in terms of textural properties of products.

5.3.8 Dynamic viscoelastic behavior

Small strain test or dynamic rheological test is widely used to measure the properties of heat-induced gelation, such as in meat protein systems (Westphalen et al., 2005). Rheograms (heating and cooling) obtained for low and normal pH meat is presented in Figure 5.6 and 5.7, respectively. The storage modulus (G') is a measure of the elastic component of the network and the loss modulus (G'') is a measure of the viscous component. Tan delta ($\tan \delta = G''/G'$) is a measure of the energy lost due to viscous flow compared to the energy stored due to elastic deformation in a single deformation cycle. Decrease in tan delta values shows the formation of better three-dimensional network (Sun and Arntfield, 2010). In the heating process, the maximum rate of increase in G' values was in the temperature range of 40°C to 55°C. The highest G' values of low (842 kPa) and normal pH (808 kPa) meat occurred at 69°C and was obtained in samples that were pressurized at 100 MPa (Figure 5.6A,B). The G' values of low (760 kPa) and normal pH (800 kPa) meat that were obtained in samples pressurized at 50 MPa were comparable to those at 100 MPa, while the lowest G' values of low (495 kPa) and normal pH (583 kPa) were obtained in samples that were pressurized at

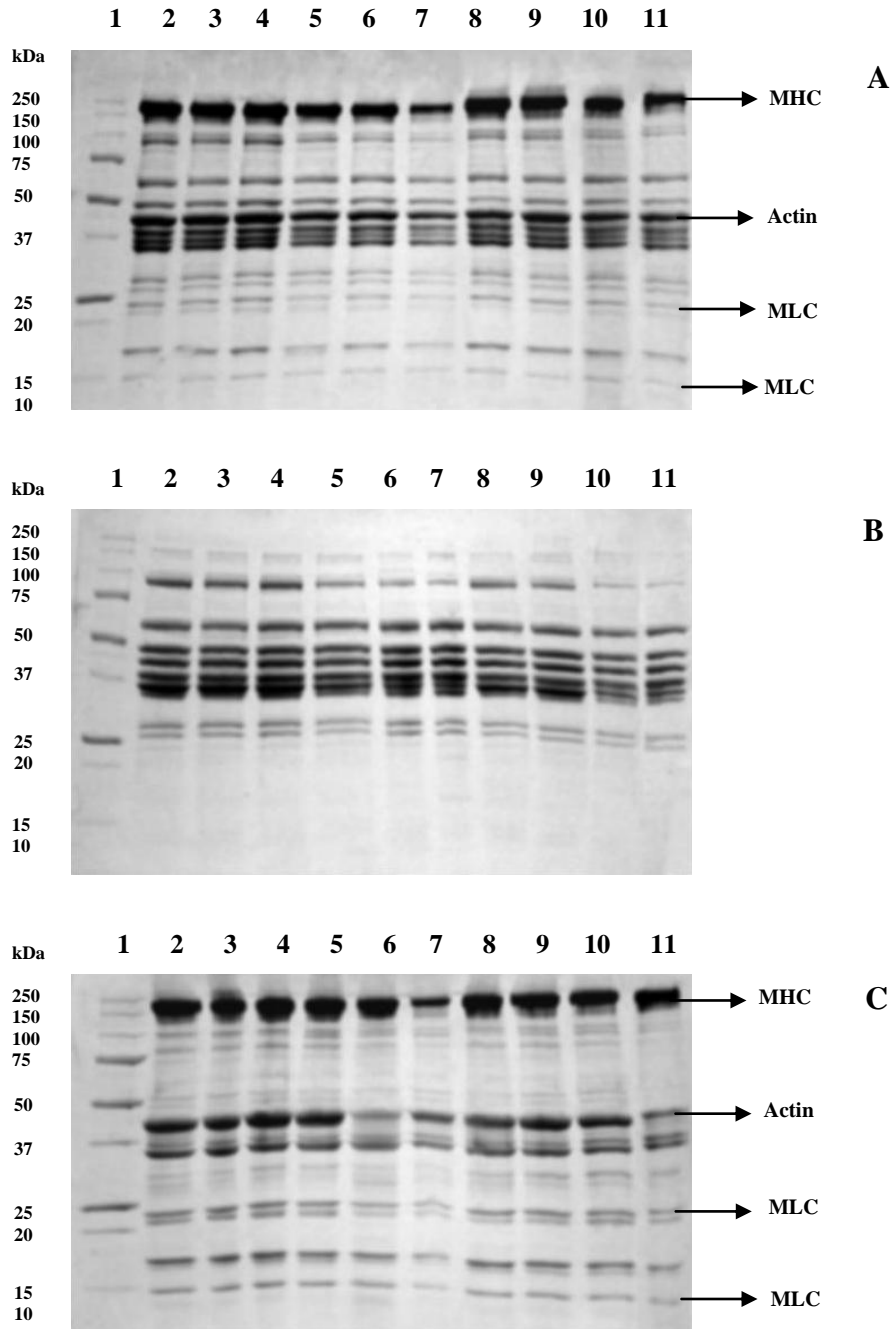


Figure 5.5. SDS-PAGE profile of total, sarcoplasmic, and myofibrillar proteins extracted from low and normal pH turkey breast meat. A: Total protein profile. B: Sarcoplasmic protein profile. C: Myofibrillar protein profile. Samples were loaded in the same order. Lane 1: standard marker; lane 2: low pH meat (control); lane 3: normal pH meat (control); lane 4: low pH meat (50 MPa); lane 5: low pH meat (100 MPa); lane 6: low pH meat (150 MPa); lane 7: low pH meat (200 MPa); lane 8: normal pH meat (50 MPa); lane 9: normal pH meat (100 MPa); lane 10: normal pH meat (150 MPa); lane 11: normal pH meat (200 MPa). MHC: Myosin heavy chains; MLC: Myosin light chains.

200 and 150 MPa, respectively. This data along with higher expressible moisture content at 150 MPa confirmed that the gel network formed in normal pH meat at this pressure level had less ability to retain water in the gel network compared to other pressure treated samples. These results suggest that low and normal pH had formed a stronger intermolecular network and had better gel forming ability in samples that were subjected to pressure at 50 and 100 MPa, compared to control samples and samples treated at 150 and 200 MPa. In low and normal pH meat, higher hydrophobicity was evident at 50 and 100 MPa, leading to more hydrophobic interactions which resulted in a better gel network. During heating, increase in G' values indicates that proteins underwent ordered aggregation and formation of a three-dimensional network with entrapment of water in the matrix. The forces responsible for gelation are hydrophobic interactions, disulphide cross bridges and hydrogen bonds (Hamann, 1992). The highest loss modulus values of low pH meat (160 kPa) were obtained in samples at 100 MPa, while in normal pH meat (152 kPa), they were obtained in samples at 50 MPa, which revealed the formation of a viscoelastic gel network (Figure 5.6C,D). Similar to storage modulus values, the lowest loss modulus values of low and normal pH meat occurred in samples at 200 and 150 MPa, respectively. During heating, tan delta values of low and normal pH meat samples at 100 MPa were the lowest compared to the other samples, which suggests that pressure at 100 MPa produced a better three-dimensional network in the two classes of meat (Figure 5.6E,F). In muscle proteins, tan delta values denote two transitions; first one at 52°C (due to denaturation of myosin) and the second one at 70°C (due to denaturation of

Table 5.3. Effect of high pressure processing on textural properties of low and normal pH meat¹

		Hardness (N)	Springiness	Chewiness	Cohesiveness	Resilience
Classes of Meat (CM) (n = 20)						
LpH		13.8 ± 1.6 ^a	0.89 ± 0.07 ^a	885 ± 100 ^a	0.71 ± 0.03 ^a	0.46 ± 0.04 ^a
NpH		13.3 ± 2.6 ^a	0.90 ± 0.06 ^a	870 ± 182 ^a	0.71 ± 0.02 ^a	0.48 ± 0.03 ^a
Treatment (T) (n = 8)						
Control		14.2 ± 1.8 ^{a,b}	0.86 ± 0.02 ^b	851 ± 94 ^{a,b}	0.68 ± 0.04 ^b	0.43 ± 0.03 ^c
50 MPa		11.7 ± 3.1 ^b	0.85 ± 0.02 ^b	714 ± 176 ^b	0.71 ± 0.02 ^{a,b}	0.46 ± 0.03 ^b
100 MPa		14.2 ± 1.2 ^a	0.86 ± 0.02 ^b	892 ± 57 ^a	0.71 ± 0.02 ^{a,b}	0.47 ± 0.02 ^b
150 MPa		13.8 ± 2.1 ^{a,b}	0.96 ± 0.06 ^a	967 ± 138 ^a	0.72 ± 0.02 ^a	0.49 ± 0.02 ^{a,b}
200 MPa		13.7 ± 1.3 ^{a,b}	0.95 ± 0.06 ^a	965 ± 76 ^a	0.73 ± 0.01 ^a	0.51 ± 0.01 ^a
Interaction (CM x T) (n = 4)						
LpH	Control	14.0 ± 1.6 ^a	0.85 ± 0.01 ^{b,c}	810 ± 55 ^{b,c}	0.67 ± 0.04 ^b	0.41 ± 0.04 ^c
	50 MPa	14.0 ± 0.6 ^a	0.84 ± 0.01 ^c	839 ± 45 ^{a,b}	0.70 ± 0.01 ^{a,b}	0.45 ± 0.06 ^{b,c}
	100 MPa	14.4 ± 1.2 ^a	0.87 ± 0.01 ^{b,c}	910 ± 51 ^{a,b}	0.71 ± 0.02 ^{a,b}	0.47 ± 0.02 ^{a,b}
	150 MPa	12.8 ± 2.7 ^{a,b}	0.94 ± 0.07 ^{a,b}	888 ± 159 ^{a,b}	0.73 ± 0.03 ^{a,b}	0.49 ± 0.03 ^{a,b}
	200 MPa	13.5 ± 1.7 ^{a,b}	0.97 ± 0.06 ^a	980 ± 85 ^{a,b}	0.73 ± 0.01 ^a	0.51 ± 0.02 ^a
NpH	Control	14.3 ± 2.2 ^a	0.88 ± 0.02 ^{a,b,c}	893 ± 115 ^{a,b}	0.70 ± 0.03 ^{a,b}	0.44 ± 0.03 ^{b,c}
	50 MPa	9.4 ± 2.8 ^b	0.86 ± 0.03 ^{b,c}	589 ± 168 ^c	0.72 ± 0.03 ^{a,b}	0.48 ± 0.03 ^{a,b}
	100 MPa	14.1 ± 1.4 ^a	0.85 ± 0.02 ^{b,c}	874 ± 64 ^{a,b}	0.71 ± 0.01 ^{a,b}	0.47 ± 0.02 ^{a,b}
	150 MPa	14.8 ± 0.8 ^a	0.97 ± 0.05 ^a	1046 ± 49 ^a	0.72 ± 0.01 ^{a,b}	0.48 ± 0.01 ^{a,b}
	200 MPa	13.8 ± 0.9 ^a	0.92 ± 0.05 ^{a,b,c}	949 ± 75 ^{a,b}	0.73 ± 0.01 ^a	0.51 ± 0.01 ^a
Source of Variation		P - Value				
CM		0.4066	0.8113	0.6249	0.5034	0.1076
T		0.0415	<0.0001	<0.0001	0.0040	<0.0001
CM x T		0.0100	0.1618	0.0030	0.5543	0.3125

¹Results are presented as means ± standard deviations. Means within column within each analysis with no common superscript differ significantly ($P < 0.05$). LpH = Low pH meat; NpH = Normal pH meat.

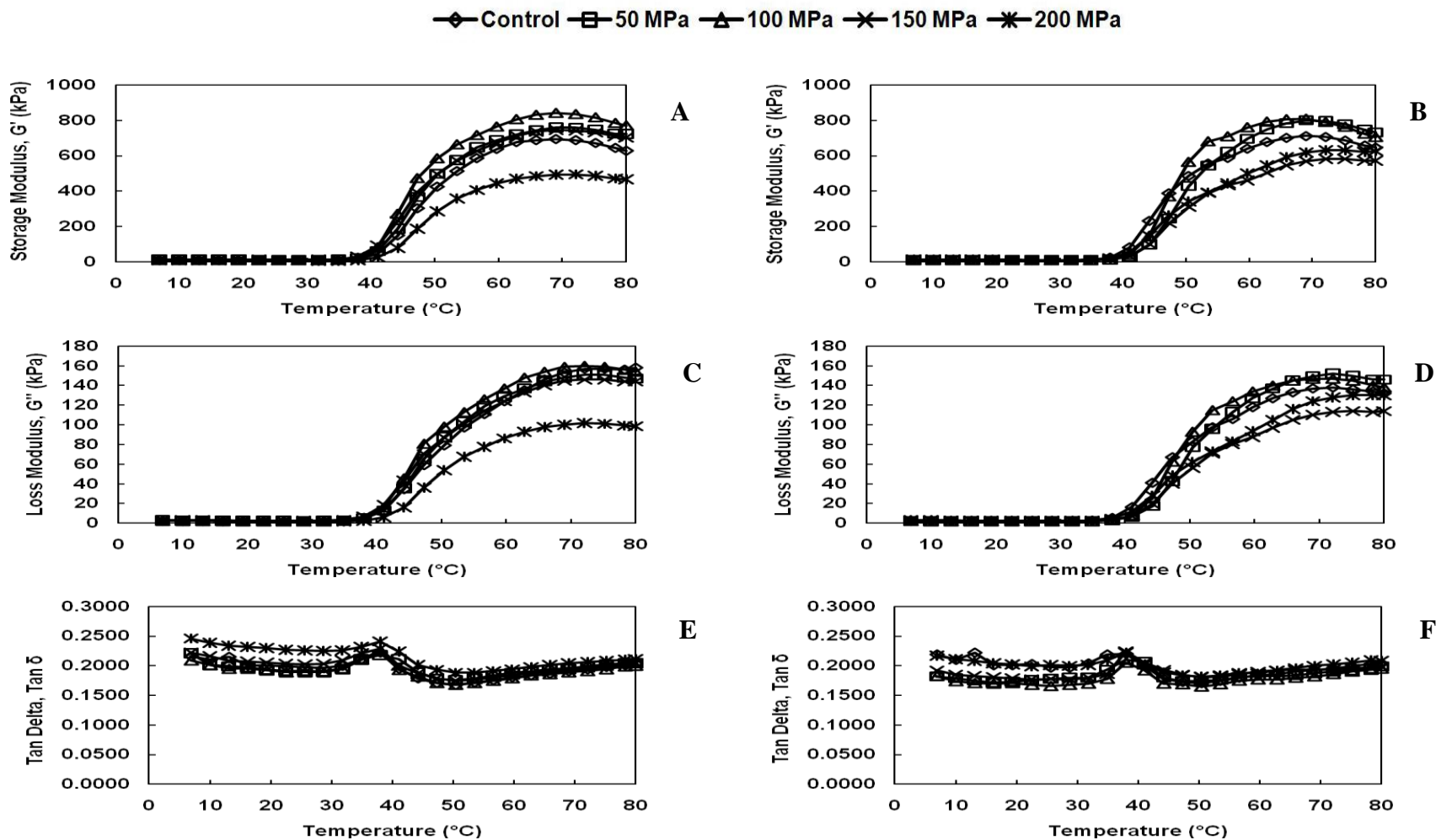


Figure 5.6. Rheograms of low and normal pH meat during heating (7°C to 80°C). A: Storage modulus (G') of low pH meat, B: Storage modulus (G') of normal pH meat, C: Loss modulus (G'') of low pH meat, D: Loss modulus (G'') of normal pH meat, E: Tan delta ($\tan \delta$) of low pH meat, F: Tan delta ($\tan \delta$) of normal pH meat.

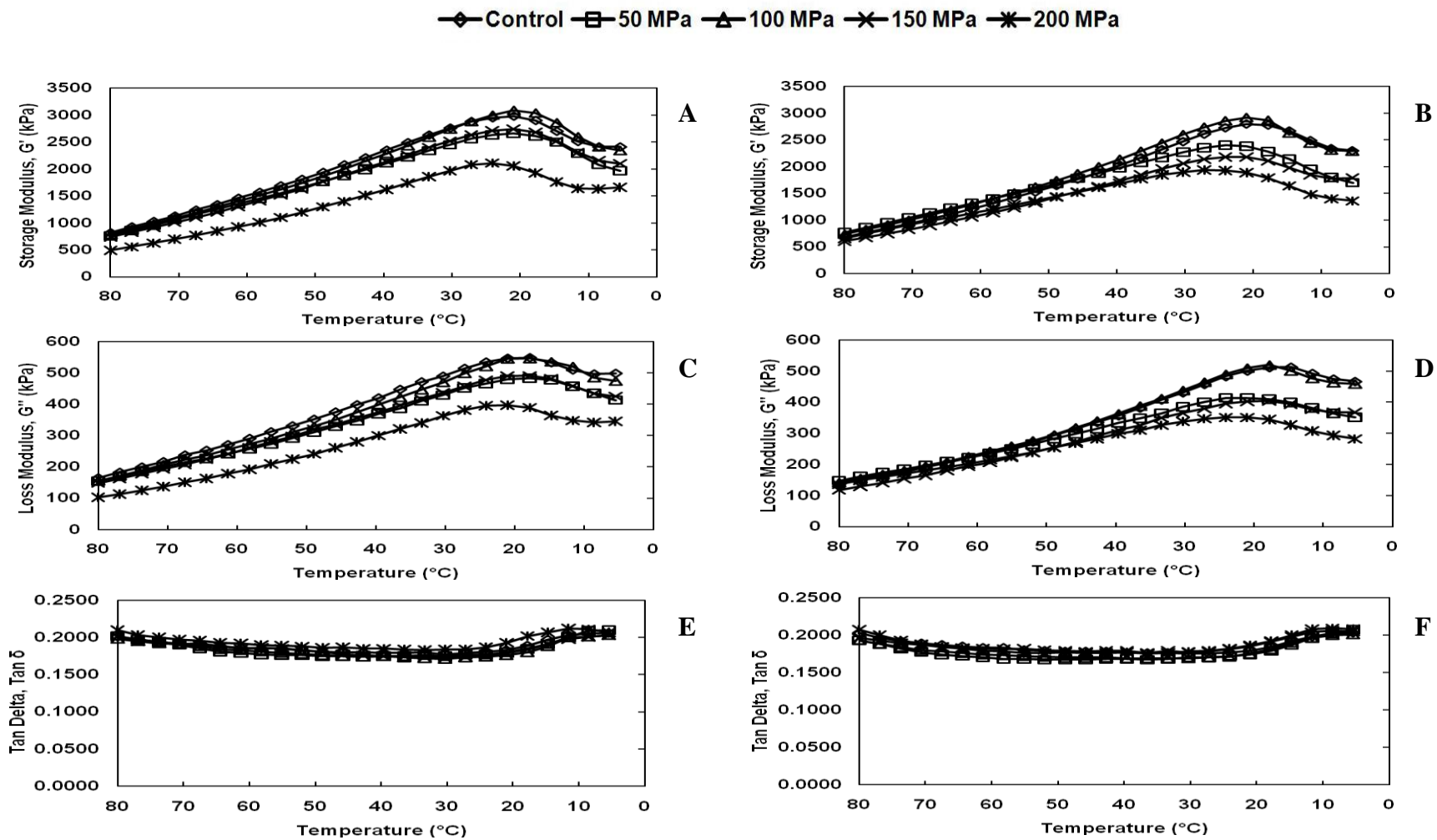


Figure 5.7. Rheograms of low and normal pH meat during cooling (80°C to 7°C). A: Storage modulus (G') of low pH meat, B: Storage modulus (G') of normal pH meat, C: Loss modulus (G'') of low pH meat, D: Loss modulus (G'') of normal pH meat, E: Tan delta ($\tan \delta$) of low pH meat, F: Tan delta ($\tan \delta$) of normal pH meat.

collagen) (Westphalen et al., 2005; Brunton et al., 2006). In this study, one major transition for low and normal pH meat was evident at 50.4°C (Figure 5.6E,F) in control and pressure treated samples, which was the temperature of myosin denaturation. This revealed that the temperature of myosin denaturation was independent of pH₂₄ and pressure treatment. In this study, the myosin denaturation temperature was similar to the one found by Omana et al. (2010), in which they have found 50.1°C in proteins from chicken dark meat. The second transition due to denaturation of collagen was not evident in the present study. This may be due to the low amount of collagen present in breast meat.

During cooling, G' and G'' values of low and normal pH meat increased until approximately 20°C, after which they slightly decreased, which indicates the formation of cross-links and rearrangement of the network structure (Figure 5.7A,B,C,D). This increase in G' and G'' values was due to the formation of hydrogen bonds, which contributed to the stability and elasticity of myosin gel networks (Hamann, 1988). Tan delta values of low and normal pH meat remained relatively constant (Figure 5.7E,F), which indicated that a stable gel had formed and the formation of hydrogen bonds during the cooling phase added strength to both the elastic and viscous components to the network (Sun and Arntfield, 2010). The data from the dynamic rheological test revealed that heating and cooling had caused an increase in gel rigidity and strengthening of the gel network, respectively.

5.4 CONCLUSION

This study revealed that functional and rheological properties of low and normal pH meat control samples were not different, except lower water holding capacity of low pH meat. In cooked and uncooked samples, pressure at 50 and 100 MPa, respectively, was found to be the best treatment to increase the WHC of low pH meat, close to the level of normal pH meat control samples. In low pH meat, pressure at 50 and 100 MPa caused more protein unfolding compared to other pressure levels, as revealed by higher sarcoplasmic and myofibrillar protein surface hydrophobicity values and reactive sulfhydryl groups, which may have contributed to improved water retention properties. Application of pressure caused an increase in total protein solubility of both low and normal pH meat. In low and normal pH meat, samples treated at lower pressure levels (50 and 100 MPa) had better gel forming ability as revealed by dynamic viscoelastic behavior. This study concludes that pressure of 50 and 100 MPa are the best treatments to improve the WHC of low pH meat.

5.5 REFERENCES

- Angsupanich, K. and D. A. Ledward. 1998. High pressure treatment effects on cod (*Gadus morhua*) muscle. *Food Chem.* 63:39-50.
- Angsupanich, K., M. Edde, and D. A. Ledward. 1999. Effects of high pressure on the myofibrillar proteins of cod and turkey muscle. *J. Agric. Food Chem.* 47:92-99.
- Barbut, S. 1993. Color measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. *Food Res. Int.* 26:39-43.
- Barbut, S. 2009. Pale, soft, and exudative poultry meat - Reviewing ways to manage at the processing plant. *Poult. Sci.* 88:1506-1512.
- Bertram, H. C., A. K. Whittaker, H. J. Andersen, and A. H. Karlsson. 2003. pH dependence of the progression in NMR T_2 relaxation times in post-mortem muscle. *J. Agric. Food Chem.* 51:4072-4078.
- Bertram, H.C. and H. J. Andersen. 2007. NMR and the water-holding issue of pork. *J. Anim. Breed. Genet.* 124:35-42.
- Brunton, N. P., J. G. Lyng, L. Zhang, and J. C. Jacquier. 2006. The use of dielectric properties and other physical analyses for assessing protein denaturation in beef Biceps femoris muscle during cooking from 5 to 85 °C. *Meat Sci.* 72:236-244.
- Chapleau, N. J. and M. I. de Lamballerie-Anton. 2003. Changes in myofibrillar proteins interactions and rheological properties induced by high-pressure processing. *Eur. Food Res. Technol.* 216:470-476.

- Cheftel, J. C. and J. Culioli. 1997. Effects of high pressure on meat: A review. *Meat Sci.* 46:211-236.
- Choi, Y. J. and J. W. Park. 2002. Acid-aided protein recovery from enzyme-rich Pacific Whiting. *J. Food Sci.* 67:2962-2967.
- Fraqueza, M. J., A. S. Cardoso, M. C. Ferreira, and A. S. Barreto. 2006. Incidence of *Pectoralis major* turkey muscles with light and dark color in a Portuguese slaughterhouse. *Poult. Sci.* 85:1992-2000.
- Gornall, A. G., J. C. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751-766.
- Hamann, D. D. 1988. Rheology as a means of evaluating muscle functionality of processed foods. *Food Technol.* 42:66-71.
- Hamann, D. D. 1992. Rheological studies of fish proteins. Pages 4-49 in International Conference and Industrial Exhibition on Food Hydrocolloids. D. D. Hamann, ed. Japanese Society of Food Hydrocolloids, Tokyo, Japan.
- Hayakawa, I., Y. Y. Linko, and P. Linko. 1996. Mechanism of high pressure denaturation of proteins. *Lebensm.-Wiss. U.-Technol.* 29:756-762.
- Hsu, K. C. and C. L. Jao. 2007. Effect of pretreatment of hydrostatic pressure on physicochemical properties of tilapia muscle protein gels induced by setting. *J. Food Sci.* 72:E73-E78.
- Hummer, G., S. Garde, A. E. García, M. E. Paulaitis, and L. R. Pratt. 1998. The pressure dependence of hydrophobic interactions is consistent with the observed pressure denaturation of proteins. *Proc. Natl. Acad. Sci.* 95:1552-1555.

- Ikeuchi, Y., H. Tanji, K. Kim, and A. Suzuki. 1992. Mechanism of heat-induced gelation of pressurized actomyosin: Pressure-induced changes in actin and myosin in actomyosin. *J. Agric. Food Chem.* 40:1756-1761.
- Iwasaki, T., K. Noshiroya, N. Saitoh, K. Okano, and K. Yamamoto. 2006. Studies of the effect of hydrostatic pressure pretreatment on thermal gelation of chicken myofibrils and pork meat patty. *Food Chem.* 95:474-483.
- Jofré, A., M. Garriga, and T. Aymerich. 2008. Inhibition of *Salmonella* sp. *Listeria monocytogenes* and *Staphylococcus aureus* in cooked ham by combining antimicrobials, high hydrostatic pressure and refrigeration. *Meat Sci.* 78:53-59.
- Kim, Y. S., J. W. Park, and Y. J. Choi. 2003. New approaches for the effective recovery of fish proteins and their physicochemical characteristics. *Fish. Sci.* 69:1231-1239.
- Ko, W. C., C. L. Jao, and K. C. Hsu. 2003. Effect of hydrostatic pressure on molecular conformation of tilapia (*Oreochromis niloticus*) myosin. *J. Food Sci.* 68:1192-1195.
- Laemelli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* 680-685.
- Lakshmanan, R., J. A. Parkinson, and J. R. Piggott. 2007. High-pressure processing and water-holding capacity of fresh and cold-smoked salmon (*Salmo salar*). *LWT* 40:544-551.
- Macfarlane, J. J. 1974. Pressure-induced solubilization of meat proteins in saline solution. *J. Food Sci.* 39:542-547.

- Marcos, B., J. P. Kerry, and A. M. Mullen. 2010. High pressure induced changes on sarcoplasmic protein fraction and quality indicators. *Meat Sci.* 85:115-120.
- Martinez, O., J. Salmeron, M. D. Guillen, and C. Casas. 2004. Texture profile analysis of meat products treated with commercial liquid smoke flavourings. *Food Control* 15:457-461.
- Messens, W., J. VanCamp, and A. Huyghebaert. 1997. The use of high pressure to modify the functionality of food proteins. *Trends Food Sci. Technol.* 8:107-112.
- Offer, G. and P. Knight. 1988. The structural basis of water-holding in meat. Pages 173-243 in *Developments in Meat Science*. R. A. Lawrie, ed. Elsevier Applied Science, London, UK.
- Ohshima, T., H. Ushio, and C. Koizumi. 1993. High-pressure processing of fish and fish products. *Trends Food Sci. Technol.* 4:370-375.
- Omana, D. A., V. Moayedi, Y. Xu, and M. Betti. 2010. Alkali-aided protein extraction from chicken dark meat: Textural properties and color characteristics of recovered proteins. *Poult. Sci.* 89:1056-1064.
- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Poulter, R. G., D. A. Ledward, S. Godber, G. Hall, and B. Rowlands. 1985. Heat-stability of fish muscle proteins. *J. Food Technol.* 20:203-217.
- Poultry Marketplace. 2010. World future outlook of the chicken meat market. http://www.agr.gc.ca/poultry/prindc2_eng.htm.

- Santos, C., L. C. Roserio, H. Goncalves, and R. S. Melo. 1994. Incidence of different pork quality categories in a Portuguese slaughterhouse: A survey. *Meat Sci.* 38:279-287.
- Sikes, A. L., A. B. Tobin, and R. K. Tume. 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Inn. Food Sci. Emerg. Technol.* 10:405-412.
- Sun, X. D. and S. D. Arntfield. 2010. Gelation properties of salt-extracted pea protein induced by heat treatment. *Food Res. Int.* 43:509-515.
- Tanaka, N., C. Ikeda, K. Kanaori, K. Hiraga, T. Konno, and S. Kunugi. 2000. Pressure effect on the conformational fluctuation of apomyoglobin in the native state. *Biochem.* 39:12063-12068.
- Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* 79:1057-1061.
- Westphalen, A. D., J. L. Briggs, and S. M. Lonergan. 2005. Influence of pH on rheological properties of porcine myofibrillar protein during heat induced gelation. *Meat Sci.* 70:293-299.
- Woelfel, R. L., C. M. Owens, E. M. Hirschler, R. Martinez-Dawson, and A. R. Sams. 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poult. Sci.* 81:579-584.
- Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.

CHAPTER 6. PROJECT SUMMARY AND INDUSTRIAL RELEVANCE

The growing consumer demand for more convenient, ready to eat meat products resulted in processors increasing the production of value-added further processed products (i.e. breaded or battered products). The pale, soft, exudative (PSE)-like meat condition has been identified in turkeys which is similar to the one observed in pigs (Owens et al., 2009). The existence of this condition is a major concern in the poultry industry because it affects meat quality attributes and thus, it has led to an estimated economic loss of at least U.S. \$200 million per year in the turkey industry (Owens et al., 2009). As a result, there is a need for processors to search for ways to utilize PSE-like meat for the production of further processed products.

The aim of the first study was to determine the biochemical and functional properties of proteins in turkey breast meat with different ultimate pH at 24 h *post mortem* (pH₂₄) in fresh and frozen conditions. In the experiments, samples were chosen at 24 h *post mortem* because the PSE-like meat condition in poultry is closely related to the extent of acidification at *post mortem* (i.e. low pH at 24 h) rather than rapid *post mortem* pH decline after slaughter which is often seen in pigs. Meat with different pH₂₄ was referred to as low, normal and high pH meat. Results in this study revealed that both low and normal pH meat had similar biochemical and functional properties indicating their similarity in the extent of protein denaturation, except for lower water holding capacity observed in low pH meat. This suggest that other factors, such as phospholipase A₂ (PLA₂) and protease enzyme activities, and proteoglycans may play a role in water retention

properties in low pH meat. High pH meat had higher protein solubility compared to low and normal pH meat and hence, expected to have better functional properties. This study revealed that the biochemical properties of proteins in low, normal and high pH meat tended to become similar after freezing. However, the proteins were susceptible to denaturation and oxidation after freezing as indicated by a decrease in Ca^{2+} -ATPase activity, total protein solubility, reactive and total sulfhydryl groups and an increase in the formation of carbonyl groups. Thus, freezing greatly affects the biochemical properties of muscle proteins.

The objective of the second study was to determine the functional, rheological, and textural properties of proteins in frozen turkey breast meat with different ultimate pH at 24 h *post mortem*. This study revealed that the emulsifying and foaming properties of the extracted sarcoplasmic and myofibrillar proteins in low, normal, and high pH meat were similar. SDS-PAGE profile of proteins from low pH meat resembles the one from that of normal pH meat. These findings revealed that low and normal pH meat had similar extent of protein denaturation. The lowest water holding capacity was found in low pH meat. These results confirmed the findings in the first study. High pH meat had better gel forming ability and folding test scores compared to low and normal pH meat. Dynamic viscoelastic behavior (DVB) showed that myosin denaturation temperature was independent of pH_{24} . Normal and high pH meat had similar hardness, springiness and chewiness values as revealed by texture profile analysis. This study indicates that high pH meat had similar or better functional properties than normal pH meat. Thus, high pH meat is suitable for further processed

products, whereas low pH meat may need additional treatment or ingredient formulations to improve its water holding capacity.

The objective of the third study was to improve functionality of low pH meat by the application of high pressure processing (HPP). Among the properties studied, emphasis was mainly focused on evaluation of water holding capacity (WHC). In the previous two studies, it was confirmed that low pH meat had the lowest water holding capacity compared to normal and high pH meat. WHC is an important property in the preparation of further processed products because it can greatly affect the yield and quality of final products. A common practice by processors to improve functional properties and yield in meat products is the addition of non-meat ingredients, such as salt and polyphosphates (Rust 1987; Claus et al., 1994). However, the incorporation of these ingredients may become a problem for consumers due to health concerns. An alternative approach to improve functional properties of meat is to use high pressure processing (HPP). One of the most important advantages of using HPP is that it allows partial replacement of added ingredients (i.e. reduced salt level) since it shows similar effect on proteins and thus, more “natural” products can be produced (Sikes et al., 2009).

The third study revealed that pressure level of 50 and 100 MPa were the best treatments to improve the water holding capacity of low pH meat in cooked and uncooked samples, respectively, which bring the level of WHC close to that of normal pH meat control samples. The improvement in WHC of low pH meat with pressure treatment at 50 and 100 MPa may be due to higher sarcoplasmic

and myofibrillar protein surface hydrophobicity values and reactive sulfhydryl groups, which helps in water retention. This study also confirmed the findings of the two previous studies in that it revealed the similarity in the extent of protein denaturation in control (unpressurized) samples of low and normal pH meat. An increase in total protein solubility was observed in both low and normal pH meat with the application of pressure. Dynamic viscoelastic behavior revealed that pressure levels at 50 and 100 MPa improved the gel forming ability of low and normal pH meat. This study concludes that the improvement of water holding capacity along with total protein solubility and gel forming ability could be achieved in low pH meat with the application of HPP. Thus, the water retention properties in PSE-like turkey meat can be enhanced by the application of high pressure processing, thereby improving product yield. An important consideration is the cost of implementing the use of high pressure processing equipment in food processing facilities. The expense of using this technology is partially mitigated by the corresponding reduction in the cost of incorporating additional ingredients (i.e. salt and polyphosphates). However, more research is needed to further understand consumer perception on high pressure treated food products. Recently, there are increasing varieties of high pressure treated food products on the market (i.e. juice, guacamole, sliced deli meats), in which HPP are utilized in an aim to improve food safety and shelf life.

6.1 REFERENCES

- Claus, J. R., J. Colby, and G. J. Flick. 1994. Processed Meats/Poultry/Seafood. Pages 106-113 in *Muscle Foods: Meat, poultry, and seafood technology*. D. M. Kinsman, A. W. Kotula, and B. C. Breidenstein, eds. Chapman & Hall, NY, USA.
- Owens, C. M., C. Z. Alvarado, and A. R. Sams. 2009. Research developments in pale, soft, and exudative turkey meat in North America. *Poult. Sci.* 88:1513-1517.
- Rust, R. E. 1987. *Science of Meat and Meat Products*. 3rd ed. J. F. Schweigert and B. S. Schweigert, eds. Food and Nutrition Press Inc., Trumbull, CT.
- Sikes, A. L., A. B. Tobin, and R. K. Tume. 2009. Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Inn. Food Sci. Emerg. Technol.* 10:405-412.

CHAPTER 7. IMPLICATIONS

The pale, soft, exudative (PSE) meat condition in pigs is primarily caused by an accelerated rate of *post mortem* glycolysis, which results from a rapid rate of pH decline and high carcass temperature (Honikel, 1987). This combination of events causes protein denaturation in the muscle leading to poor meat quality (Santos et al., 1994). Several studies seem to indicate that PSE-like condition in poultry is more related to the extent of muscle acidification (i.e. low pH at 24 h) rather than the fast drop of pH after slaughter (Wilkins et al., 2000; Petracci et al., 2004; Fraqueza et al., 2006).

Generally, it is hypothesized that ultimate pH leads to different extent of protein denaturation, which in turn affects functional properties of proteins. Previous researchers have demonstrated that broiler and turkey breast had lower protein solubility in PSE meat compared to normal meat (Pietrzak et al., 1997). Studies in chicken and turkey revealed that low muscle pH is associated with reduced protein functionality (i.e. decreased water holding capacity and weaker gel formation) (Barbut, 1997; Zhang and Barbut, 2005). This led to the speculation that myosin denaturation and/or insolubility are contributing factors in the development of PSE in turkey breast muscle (Pietrzak et al., 1997). However, Van Laack and Lane (2000) and Van Laack et al. (2000) showed that avian myofibrillar proteins are relatively resistant to *post mortem* protein denaturation in contrast to mammalian proteins, indicating that factors other than protein denaturation are responsible for the low WHC observed in PSE-like poultry meat. The results in Chapter 3, 4 and 5 concurs with the findings of these studies which

showed that low pH turkey breast meat are in fact relatively resistant to *post mortem* myosin denaturation. Low and normal pH meat did not differ by each other in terms of biochemical properties of proteins. The lower WHC as evident in low pH turkey breast meat reveals that the development of PSE-like condition may not be directly associated with protein denaturation. The reason for the lower water holding capacity of PSE type of meat in poultry needs further investigation. Future work can be focused on studying the role of proteoglycans, which may be of importance in determining water retention properties in meat (Velleman 1998, 2000). Recent studies conducted in poultry and pigs (Soares et al., 2003; Chen et al., 2010) revealed that low *post mortem* pH values are associated with increased phospholipase A₂ (PLA₂) enzyme activities, which can cause disruption of muscle cell membrane; hence contributing to exudative loss from meat. Excessive PLA₂ enzyme activity causes increased membrane blebbing in mitochondria and in the sarcoplasmic reticulum which induces release of Ca²⁺. Thus, elevated calcium concentration in the sarcoplasm may increase protease enzyme activities (i.e. calpain), thereby affecting the integrity of muscle fiber structure and thus impairing meat protein functionality (Wilhelm et al., 2010). The role of these enzymes in water holding capacity of PSE-like turkey meat need to be further investigated.

Currently, details on the characteristics and occurrences of dark, firm, dry (DFD)-like turkey are not available. The results in Chapter 3, 4 and 5 reveals that the DFD condition in turkey seems not to be a major concern in terms of functionality since high pH turkey breast meat has similar or better functional

properties compared to normal pH meat. Results also revealed that the biochemical properties of proteins in low, normal, and high pH meat tended to become similar after freezing. However, there was reduction in functionality compared to that of samples in fresh condition, which was due to protein denaturation and protein oxidation.

Knowledge of the protein characteristics in the different types of meat with different ultimate pH will help to modify process parameters while PSE-like meat is used as raw material. High pressure processing has the potential to improve important functional properties, including water holding capacity, protein solubility, and gel forming ability. In conclusion, the improvement of water retention properties in PSE-like turkey meat is possible by application of HPP, thereby increasing the suitability of this type of meat in processed foods.

7.1 REFERENCES

- Barbut, S. 1997. Problem of pale soft exudative meat in broiler chickens. *Br. Poult. Sci.* 38:355-358.
- Chen, T., G. Zhou, X. Xu, G. Zhao, and C. Li. 2010. Phospholipase A₂ and antioxidant enzyme activities in normal and PSE pork. *Meat Sci.* 84:143-146.
- Fraqueza, M. J., A. S. Cardoso, M. C. Ferreira, and A. S. Barreto. 2006. Incidence of *Pectoralis major* turkey muscles with light and dark color in a Portuguese slaughterhouse. *Poult. Sci.* 85:1992-2000.
- Honikel, K. O. 1987. Influence of chilling on meat quality attributes of fast glycolysing pork muscles. Pages 273-283 in *Evaluation and Control of Meat Quality in Pigs*. P. V. Tarrant, G. Eikelenboom, and G. Monin, eds. Martinus Nijhoff, Dordrecht, Netherlands.
- Petracci, M., M. Betti, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. *Poult. Sci.* 83:2086-2092.
- Pietrzak, M., M. L. Greaser, and A. A. Sosnicki. 1997. Effect of rapid rigor mortis processes on protein functionality in pectoralis major muscle of domestic turkeys. *J. Anim. Sci.* 75:2106-2116.
- Santos, C., L. C. Roserio, H. Goncalves, and R. S. Melo. 1994. Incidence of different pork quality categories in a Portuguese slaughterhouse: A survey. *Meat Sci.* 38:279-287.
- Soares, A. L., E. I. Ida., S. Miyamoto, F. J. Hernández-Blazquez, R. Olivo, J. W. Pinheiro, and M. Shimokomaki. 2003. Phospholipase A₂ activity in poultry PSE, pale, soft, exudative meat. *J. Food Biochem.* 27:309-320.

- Van Laack, R. L. J. M. and J. L. Lane. 2000. Denaturation of myofibrillar proteins from chicken as affected by pH, temperature, and adenosine triphosphate concentration. *Poult. Sci.* 79:105-109.
- Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D. Loveday. 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* 79:1057-1061.
- Velleman, S. G. 1998. What's outside the cell and how does it affect avian bone and muscle formation? Pages 71-88. U.S.A.
<http://www.poultryscience.org/pba/1952-2003/1998/1998%20Velleman.pdf>
- Velleman, S. G. 2000. The role of the extracellular matrix in skeletal development. *Poult. Sci.* 79: 985-989.
- Wilhelm, A. E., M. B. Maganhini, F. J. Hernandez-Blazquez, E. I. Ida, and M. Shimokomaki. 2010. Protease activity and the ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. *Food Chem.* 119:1201-1204.
- Wilkins, L. J., S. N. Brown, A. J. Phillips, and P. D. Warriss. 2000. Variation in the colour of broiler breast fillets in the UK. *Br. Poult. Sci.* 41:308–312.
- Zhang, L. and S. Barbut. 2005. Rheological characteristics of fresh and frozen PSE, normal and DFD chicken breast meat. *Br. Poult. Sci.* 46:687-693.