Application of Proteomics in Understanding Pale Soft and Exudative Condition in Broiler Breast Meat

Monil Ajitbhai Desai

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Application of proteomics in understanding pale soft and exudative condition in broiler breast meat

By

Monil Ajitbhai Desai

A Dissertation
Submitted to the Faculty of Mississippi State University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Science and Technology in the Department of Food Science Nutrition and Health Promotion

Mississippi State, Mississippi

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2015
Application of proteomics in understanding pale soft and exudative condition in broiler breast meat

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This experiment was conducted to determine the differences in meat quality (cooking loss and shear force), descriptive sensory characteristics, consumer acceptance, and whole muscle proteomes between normal and Pale, Soft, and Exudative (PSE) broiler breast meat. Male Hubbard × Cobb 500 birds (n = 1,050) were raised in commercial houses. Prior to harvest, a sample of the broilers (n = 900) were subjected to short-term stress (38 °C for 2 h), and the remaining broilers (n = 150) were maintained at control conditions (21 °C for 2 h). From the stressed and control condition broilers, breast samples were characterized by pH24 and L*24 as normal (pH24 5.8-6.2, L*24 45-55) or PSE (pH24 5.4-5.7, L*24 55-65). Normal chicken breast meat had lower shear force values than PSE meat (P < 0.05). Based on sensory descriptive analysis, normal cooked chicken breast was more tender and juicier than PSE breast meat (P < 0.05). Consumer sensory analysis results indicated that 81% of consumer panelists liked normal breast meat whereas 62% of the panelists liked PSE breast meat. Whole muscle proteome profiling identified fifteen differentially abundant proteins (P < 0.05) in normal and PSE meat samples. Actin alpha, myosin heavy chain, phosphoglycerate kinase, creatine kinase M
type, beta-enolase, carbonic anhydrase 2, proteasome subunit alpha, pyruvate kinase, and malate dehydrogenase were over-abundant in PSE meat whereas phosphoglycerate mutase-1, alpha-enolase, ATP-dependent 6-phosphofructokinase, and fructose 1, 6-bisphosphatase were over-abundant in normal meat. In addition, normal and PSE broiler breast meat were sampled from commercial plants and evaluated for meat quality attributes (pH, color, cooking loss, and tenderness) and their whole muscle proteome. Normal chicken breast meat had lower shear force values than PSE meat ($P < 0.05$). Proteome analysis revealed five differentially abundant proteins ($P < 0.05$) between the normal and PSE chicken breast samples. Glycolytic enzymes (beta-enolase and fructose-bisphosphate aldolase C) were over-abundant in PSE breast meat. Myofibrillar protein (myosin heavy chain) was over-abundant in PSE breast meat. In conclusion, results indicated that differences in proteome abundance could be related to the meat quality differences between normal and PSE breast meat.
DEDICATION

I would like to dedicate this dissertation to my dear parents Mr. Ajit Desai and Mrs. Sangita Desai.
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CHAPTER I
INTRODUCTION

Due to changes in consumer eating and purchasing patterns, there has been a shift in how chicken products are marketed. In the 1960’s, over 80% of chicken broilers were marketed as whole carcasses, and only 2% were sold as further processed products. In contrast, in 2011, 12% of chicken broilers were sold as whole carcasses, 42% as cut up parts, and 46% as further processed products (MacDonald, 2014). Moreover, the increase in demand for cut-up or deboned chicken parts has resulted in meat quality problems that are related to water holding capacity, appearance, and texture (Petracci & Cavani, 2012). Pale, Soft, Exudative (PSE) meat has a lighter appearance, a softer texture, lower water holding capacity, excessive yield losses, and formation of soft gels when compared to normal meat (Alvarado & Sams, 2003; Barbut, Zhang, & Marcone, 2005; Owens, Hirschler, McKeel, Martinez-Dawson, & Sams, 2000). It has been estimated that the production of PSE-like breast meat results in approximately 200 million dollars of revenue loss for the poultry industry annually (Owens, Alvarado, & Sams, 2009; Petracci & Cavani, 2012). Moreover, it has been reported that approximately 5-40% of poultry products in the United States are PSE, which is dependent on season of the year (Owens et al., 2009).

Lean broiler meat contains approximately 20% protein and 75% water, which makes proteomic methods useful in understanding the relationship between muscle
proteins and meat quality (Remignon, Molette, Babile, & Fernandez, 2006). Two-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has been applied in meat quality research to understand growth and development, postmortem metabolism, the role of calpains in tenderness, and water holding capacity (Bendixen, 2005; Görg, Weiss, & Dunn, 2004). Proteomics has been applied in poultry research to understand the role of diet on broiler growth and meat quality (Paredi, Raboni, Bendixen, de Almeida, & Mozzarelli, 2012). In addition, researchers have related muscle proteomes to the growth and development of laying hens (Doherty et al., 2004). Furthermore, researchers analyzed the proteomes from fast-glycolyzing and normal glycolyzing turkeys, and suggested that the differences in proteomes were indicative of differences in meat quality (Molette, Réminignon, & Babilé, 2005). We have previously demonstrated the use of proteomic methods in understanding the red color meat quality defect in channel catfish (Ictalurus punctatus) fillets. However, proteomics has not been utilized to characterize the whole muscle proteome of PSE chicken breast meat. Therefore, the major focus of this study was to identify the proteome differences between normal and PSE chicken breast meat and to determine their relationship to meat quality.

In the first part of this study, the objectives included: (1) to evaluate the differences in cooking loss and shear force of normal and PSE broiler breast meat; (2) to determine differences in descriptive sensory characteristics and consumer acceptance of normal and PSE broiler breast meat; and (3) to correlate the meat quality parameters to the differentially abundant muscle proteome components in normal and PSE broiler breast meat. In the second part of this study, the objectives were: (1) to characterize the
whole muscle proteomes of normal and PSE broiler breast meat from a commercial processing plant; and (2) to evaluate the differences in meat quality traits (pH, color, cooking loss, and shear force) of normal and PSE broiler breast meat from a commercial processing plant.
CHAPTER II
LITERATURE REVIEW

2.1 Poultry industry

A recent report from the American Meat Institute (AMI) states that the poultry meat industry is the largest segment of United States agriculture (American Meat Institute, 2013). Per capita consumption of poultry has increased substantially since the 1970’s throughout the United States and the world (Magdelaine, Spiess, & Valceschini, 2008; Petracci, Bianchi, & Cavani, 2009). Chicken consumption has increased from 18.2 kg/year per person in 1970 to 38.2 kg/year per person in 2011, and turkey consumption has increased from 3.7 kg/year per person in 1970 to 7.3 kg/year per person in 2011 (American Meat Institute, 2013). According to the USDA, the combined production value of eggs, turkeys, and chickens was $44.1 billion in 2013, an increase of 8 percent from $38.2 billion in 2012 (NASS, USDA, 2014).

Increased demand for poultry consumption has resulted in broiler production with increased growth rate, feed efficiency, breast muscle size, and reduced abdominal fatness (Petracci & Cavani, 2012). However, these recent changes in poultry production have contributed to live animal stress, and the potential for meat quality problems. Due to changes in consumer eating and purchasing patterns, there is a shift in the way chicken products are marketed today. In the 1960’s, over 80% of broilers were marketed as whole carcasses, and only 2% as further processed products. However, in 2011, 12% of chicken
broilers were sold as whole birds, 42% as cut up parts, and 46% as further processed products (MacDonald, 2014). Moreover, the increase in demand for cut-up or deboned parts has resulted in meat quality problems that are related to water holding capacity, appearance, and texture (Petracci & Cavani, 2012). Poultry meat quality defects include Pale, Soft, and Exudative (PSE or PSE-like) breast meat, intramuscular connective tissue defects, green muscle disease, and woody breast meat. It has been estimated that the production of PSE-like breast meat results in approximately 200 million dollars in annual revenue loss for the poultry industry (Owens et al., 2009; Petracci & Cavani, 2012).

2.2 Conversion of muscle to meat (biochemical, physiological, and structural changes)

During the conversion of muscle to meat, a series of postmortem (molecular, biochemical, physiological, and structural) changes take place in the muscle (Samuel, 2009). After the death of an animal due to exsanguination (blood loss) and depletion of oxygen, the muscle tissue still continues to produce adenosine triphosphate (ATP) through anaerobic glycolysis (Aberle et al., 2001). After exsanguination, the blood circulatory system no longer functions to remove metabolites from the tissues, so lactic acid accumulates in the muscle tissues due to glycogen breakdown under anaerobic conditions that results in a decreased muscle pH (Aberle et al., 2001; Berri, 2000; Sams, 1999b). Lactic acid accumulation occurs due to the breakdown of adenosine triphosphate (ATP) and phosphocreatine (PCr) under anaerobic conditions. At 15 min postmortem, the pH of the chicken muscle is approximately 6.2-6.5 and has a final pH of approximately 5.8-6.0 at 24 h postmortem (Berri et al., 2005; Fletcher, 1999; Kijowski & Niewiarowicz,
1978; Van Laack, Liu, Smith, & Loveday, 2000). This process is referred to as the conversion of muscle to meat (Aberle et al., 2001). Postmortem, the muscle continues using ATP to dissociate actin and myosin (Hamm, 1982). Over time, the ATP concentration becomes insufficient which leads to permanent actomyosin bonds that causes myofibrillar contraction and muscle stiffness (Berri, 2000; Samuel, 2009). Stiffening of the muscle is referred to as the onset of rigor mortis which occurs when approximately 60% of ATP is used up by the muscles (Newbold, 1966). Postmortem, the rigor process is affected by ATP and calcium ion (Ca\(^{2+}\)) concentration within the muscle. Ca\(^{2+}\) ions are essential for muscle contraction since these ions regulate actin and myosin interaction, and are pumped out of cytosol into the sarcoplasmic reticulum using Ca\(^{2+}\) ion pumps. Completion of rigor mortis is affected by several factors such as species, muscle fiber type, temperature, rate of glycolysis, and the extent of struggle at the time of death (Greaser, 1986).

Poultry muscle fiber types are classified into three main types: Red (Type I/Slow-twitch), Intermediate (Type IIa), and White (Type IIb/Fast-twitch) (Berri, 2000; Foegeding, Lanier, & Hultin, 1996). Red muscle fibers are slow-contracting oxidative (SOG), intermediate muscle fibers are fast-contracting oxidative and glycolytic (FOG), and white muscle fibers are fast-contracting glycolytic (FG) (Solomon, van Laack, & Eastridge, 1998). Muscles with a high proportion of red fibers such as thighs are myoglobin rich and have greater capacity for aerobic (oxidative) metabolism that leads to fatigue-resistance, whereas muscles with a greater proportion of white fibers such as breast muscles have less myoglobin, have a greater capacity for anaerobic (glycolytic) metabolism, and are fast-fatiguing and therefore used for brief bursts of activity.
Rate of pH decline and rigor mortis development is greater in poultry breast muscle because of the increased amount of glycolytic white fibers than in leg muscle with oxidative red muscle fibers (Berri, 2000; Dransfield & Sosnicki, 1999). If poultry meat has a pH of 6.4 at 45 min postmortem, it results in Dark, Firm and Dry (DFD) poultry meat (Viljoen, De Kock, & Webb, 2002; Warriss, 2000). However, a pH lower than 6.0 at 15 min postmortem in poultry breast muscle results in the Pale, Soft and Exudative (PSE) condition (Barbut et al., 2005; Swatland, 2008; Warriss, 2000). PSE meat is paler in color and has poor water-holding capacity in comparison to normal meat and 5-40% of poultry products in the United States are PSE (Owens et al., 2009). Thus, understanding the biochemical changes that occur during the conversion of muscle to meat provides information on how PSE meat is produced.

2.3 Poultry meat quality parameters

Biochemical changes during the conversion of muscle to meat impacts meat quality. Poultry meat quality is characterized by sensory (appearance, tenderness, juiciness, firmness, color, flavor), physical (drip loss, cook loss, water-holding capacity), chemical (pH, shelf life etc,) and microbiological measurements (Aberle et al., 2001; Allen, Fletcher, Northcutt, & Russell, 1998; Cross, Durland, & Seideman, 1986; Duclos, Berri, & Le Bihan-duval, 2007). Several factors such as growth rate, age, sex of the animal, genotype, and pre-slaughter stress conditions impact muscle development and meat quality (Debut et al., 2005; Duclos et al., 2007; Sonaiya, Ristic, & Klein, 1990).
2.3.1 **pH**

Chicken breast pH at 15 min postmortem (pH\textsubscript{15}) between 6.2 to 6.5 and a 24 hour pH (pH\textsubscript{u}) of approximately 5.8 is considered normal (Berri et al., 2005; Fletcher, 1999; Kijowski & Niewiarowicz, 1978; Van Laack et al., 2000). Research has shown that pH\textsubscript{u} values influences cooking yield, protein extraction, and protein functionality (Daum-Thunberg, Foegeding, & Ball, 1992). pH values of approximately 6.0 have greater protein functionality, solubility, and salt extraction in comparison to poultry meat with a pH less than 5.7 (Van Laack et al., 2000). Muscle pH affects meat quality attributes such as tenderness, water holding capacity, cook loss, juiciness, and microbial stability (Fletcher, 2002). pH values less than 5.7 are associated with pale color and lower water holding capacity (Van Laack et al., 2000). A rapid decline in pH postmortem contributes to the production of PSE meat. When the pH\textsubscript{15} of breast meat falls below 6.0 while the muscle is still warm, protein denaturation occurs that results in poor water holding capacity and pale color in poultry meat (Allen, Russell, & Fletcher, 1997).

2.3.2 **Color**

Appearance is the single most important factor with respect to the selection or rejection of a meat or poultry product (Fletcher, 2002). Poultry meat color is objectively measured using a chroma meter based on the International Commission on Illumination (CIELAB) system in terms of lightness (\(L^*\)), redness (\(a^*\)), and yellowness (\(b^*\)) (Bianchi & Fletcher, 2002). CIE (Commission Internationale de l’Eclairage or the International Commission on Illumination) is the main international organization concerned with color measurement (Loughrey, 2005). Chroma meters generally consist of a CIE adopted Illuminant D\textsubscript{65} and a CIE 2\(^0\) standard observer (Wrolstad & Smith, 2010). The measuring
head of the chroma meter is divided into the measuring part which measures the light reflected from the specimen, and the illumination part which measures the light from the illumination light source. Data for amount of light reflected or transmitted is sent to a processor and converted to CIE tristimulus values (Wrolstad & Smith, 2010). Raw poultry meat is pale tan to pink whereas cooked poultry meat has a tan to gray color (Fletcher, 2002). Poultry meat color is influenced by several factors such as heme pigments (myoglobin, hemoglobin, cytochrome C, and their derivatives), pre-slaughter factors (genetics, feed, feed withdrawal, stress), slaughter, chilling, and further processing (stunning techniques, salt, phosphates) (Froning, 1995). In poultry, myoglobin (heme pigment) is the sarcoplastic fraction which accounts for 80 to 90% of the total pigment whereas hemoglobin is the blood pigment that accounts for 20 to 30% of color in a properly bled carcass (Froning, 1995; Hedrick, Aberle, Forrest, Judge, & Merkel, 1994; Lawrie, 1998). Poultry meat color is affected by myoglobin content, chemical state of the heme structure, and meat pH (Fletcher, 2002; Lawrie, 1998). Myoglobin content varies based on species, muscle, and animal age (Daigle, 2005). Poultry thigh and leg meat exhibit dark red color due to higher myoglobin content whereas breast meat has a pink color and is referred to as white meat (Daigle, 2005; Hedrick et al., 1994). Zhang & Barbut, (2005) reported that higher lightness ($L^*$) values and lower pH values are indicators of PSE chicken breast meat. Rathgeber, Pato, Boles, & Shand, (1999) studied the effect of rapid postmortem pH decline and delayed chilling on turkey breast meat quality. Results from the study indicated that delayed chilling resulted in lighter (higher $L^*$), redder (higher $a^*$), and more yellow (higher $b^*$) breast meat than samples from carcass halves that were chilled immediately. In the present study, the application of
proteomics was used to elucidate the protein expression differences between normal and PSE chicken breast meat. In particular, sarcoplasmic protein expression differences in normal and PSE chicken breast meat might be helpful in explaining the color differences between normal and PSE chicken breast meat.

2.3.3 Water Holding Capacity (WHC)

Water-holding capacity (WHC) is defined as “the ability of muscle to retain water when exposed to external forces such as cutting, heating, grinding or pressing” (Hedrick et al., 1994). WHC is correlated with meat color, texture, and juiciness (Aberle et al., 2001). Meat tissue contains approximately 75% water which exists in either the free, immobilized, or bound form (Hedrick et al., 1994; Huff-Lonergan & Lonergan, 2005). Bound water makes up approximately 5% of the total water whereas immobilized water accounts for 95% of the remaining water (Belitz, Grosch, & Schieberle, 2004). WHC is affected by several factors such as production of lactic acid, loss of ATP, onset of rigor mortis, and structural changes in the muscle cell (Aberle et al., 2001; Huff-Lonergan & Lonergan, 2005). Rapid pH decline while the carcass is at a high temperature (i.e. greater than 35°C) results in the denaturation of myofibrillar proteins, which leads to purge or drip loss and the production of PSE meat. Several researchers have reported a negative correlation between $L^*$ (lightness values) and WHC (i.e. the higher the $L^*$ values, the lower the WHC (Barbut, 1997a, 1997b; McCurdy, Barbut, & Quinton, 1996; Sosnicki, Greaser, Pietrzak, Pospiech, & Sante, 1998). WHC is measured in terms of drip loss and cooking loss in poultry meat. Drip loss is measured as loss of water from a muscle during storage whereas cooking loss is reported as the amount of water loss after cooking meat at 70°C for approximately 30 min (Honikel, 1998).
2.3.4 Sensory parameters (Tenderness, Juiciness, and Flavor)

Tenderness is an important meat quality attribute that is affected by several postmortem factors such as temperature, sarcomere length, proteolysis, breed, strain, and animal age (Lawrie, 1998; Maltin, Balcerzak, Tilley, & Delday, 2003). Poultry meat tenderness is also impacted by connective tissue maturity and the contractile state of the myofibrillar proteins (Fletcher, 2002). Biochemical changes during the conversion of muscle to meat impact tenderness. Deboning breast meat prior to the completion of rigor mortis results in tougher meat due to muscle fiber contraction and muscle shortening (Fletcher, 2002). Tenderness is most often evaluated using a Warner Bratzler or Allo-Kramer shear force attachment which measures tenderness in terms of peak force and total energy (Fernandez et al., 2001). Poultry meat sensory properties are evaluated in terms of appearance (shape and color), flavor (odor and taste), juiciness, and tenderness (Grashorn, 2010). Juiciness contributes to mouth feel and is indirectly related to texture and flavor (Lawrie, 1998). Juiciness is impacted by the pH of the meat. Lower meat pH (pH < 5.7) results in lower WHC, higher cook loss, lower yield, and a less juicy product (Van Laack et al., 2000).

2.4 Factors that contribute to the production of PSE meat

Since the 1960’s, the poultry industry has increased meat production due to an increased population worldwide and increased per capita consumption of broiler meat. PSE is a quality defect in broiler breast meat that originates during rigor mortis when carcasses experience acidic conditions within their muscles when the muscle temperature is still high (Solomon et al., 1998). Breed, sex, species, pre-slaughter, and post slaughter handling of animals are some of the factors that lead to the production of PSE meat.
PSE meat results from ante-mortem stress and/or genetic traits within the live animal (breed, animal growth rate, and muscle fiber type). Antemortem stress factors include feed withdrawal, environmental changes, overcrowded crates, handling of birds, and stressful transportation (Backstrom & Kauffman, 1995; Cassens, Marple, & Eikelenboom, 1975; D’Souza, Dunshea, Warner, & Leury, 1998; Grashorn, 2010; McKee & Sams, 1998; Offer & Trinick, 1983; Owens & Sams, 2000). Antemortem stress factors ultimately cause accelerated postmortem glycolysis that leads to a lower initial pH (Dodge & Peters, 1960; Hedrick et al., 1994; Lawrie, 1985). One of the major antemortem stress factors includes heat (chronic or acute) stress. Previous studies have shown the production of PSE meat is more prevalent during the summer season (Barbut et al., 2008; McCurdy et al., 1996; McKee & Sams, 1997; Owens et al., 2009). McCurdy et al., (1996) studied the seasonal effect of PSE occurrence in young turkey breast meat by measuring pH, color, water holding capacity, and cooking loss. Results from the study suggested that lightness ($L^*$ value) was highest, lowest, and intermediate during the summer, winter, and spring seasons, respectively. McKee & Sams (1997) examined the effect of seasonal stress on rigor development and the incidence of PSE turkey meat. Results indicated that the meat from heat stressed birds was paler and had increased drip loss in comparison to normal birds. Petracci, Betti, Bianchi, & Cavani (2004) reported that PSE meat was more prevalent in the summer (15.5%) than in the winter (2.7%). Petracci et al., (2009) indicated that the occurrence of PSE meat was as high as 40% during hot weather. In addition, genetic factors can also impact the ability of birds to overcome stress and inhibit the production of PSE meat. Genetic factors include breed, animal growth rate, and muscle fiber type (Adzitey &
Nurul, 2011). Genetic improvements have led to larger carcasses that have been produced in a short period of time with more muscling which can also contribute to the production of PSE meat.

Muscle fiber type influences the development of PSE meat in poultry. Poultry breast muscles have a higher proportion of white muscle fibers, which makes it more susceptible to the production of PSE meat due to a greater dependence on anaerobic/glycolytic metabolism when compared to red muscle fibers such as poultry thigh (Solomon et al., 1998). Because of their muscle fiber (entirely glycolytic Type II-b) composition, postmortem glycolysis and muscle pH decline is much faster in poultry breast muscles than in red meat species (Addis, 1986; Barbut et al., 2008; Ma & Addis, 1973; Wiskus, Addis, & Ma, 1976). Subjecting broilers to short-term or acute stress prior to slaughter can cause a rapid buildup of lactic acid in the muscles both prior to and immediately after slaughter (Adzitey & Nurul, 2011). Postmortem, the breast muscle shifts to anaerobic metabolism so glycogen is converted to lactic acid within the breast muscle, which contributes to a rapid pH decline (Petracci & Cavani, 2012). Elevated muscle temperature coupled with an acidic pH causes a rapid breakdown in proteins (myofibrillar), thus contributing to the production of poultry that is soft and exudative. Myoglobin, the predominant sarcoplasmic protein responsible for color, is also either denatured or adsorbed onto myofibrillar proteins (Kauffman & Marsh, 1987), resulting in an increased reflectance of light on the meat surface and thus causing an increase in paleness, which negatively impacts product quality and consumer acceptance (Owens et al., 2009).
2.5 Pale, Soft and Exudative (PSE) meat in the poultry industry

In swine, the occurrence of PSE meat was first reported in the early 1960’s, whereas in poultry it was first observed in the early 1970’s (Aberle, Stadelman, Zachariah, & Haugh, 1971; Brisky & Wismer-Pedersen, 1961; Vanderstoep & Richards, 1974). In poultry, a combination of rapid postmortem glycolysis and high temperature (above 35°C) can lead to the production of PSE poultry meat (Alvarado & Sams, 2003; Pietrzak, Greaser, & Sosnicki, 1997). Rapid pH decline along with high postmortem temperature leads to myofibrillar and sarcoplasmic protein denaturation, and consequently the production of PSE meat (Briskey & Wismer-Pedersen, 1961; Kissel, Soares, Rossa, & Shimokomaki, 2009). In the poultry industry, PSE meat has been observed both in turkey and broiler breast meat (Barbut, 1997a, 1997b). In the past, several studies have focused on determining the prevalence of PSE meat in the poultry industry (Barbut, 1996, 1997a, 1997b; McCurdy et al., 1996; Owens et al., 2000; Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams, 2002; Woelfel & Sams, 2001). McCurdy et al., (1996) indicated that PSE meat in young turkey flocks can range from 5 to 30%. Barbut (1997a), using a cut-off point of $L^* \geq 52$, reported an incidence of PSE meat between 5-40% in commercial mature turkey hens. Similarly, Barbut (1998) reported a PSE incidence between 0-28% in commercial broiler chickens using a cut-off point of $L^* > 49$. Owens et al., (2000) reported a PSE incidence of 40% in a commercial turkey processing plant using a cut-off point of $L^* > 53$. Woelfel et al., (2002) reported 47% prevalence of pale broiler fillets in a commercial processing plant ($L^* > 54$) and Petracci et al., (2004) reported a 2.7-15.5% occurrence of PSE meat ($L^* > 56$) for broiler breast fillets during processing in Italy.
PSE meat is associated with having a lighter appearance, a softer texture, lower water holding capacity, excessive yield losses, and formation of soft gels (Alvarado & Sams, 2003; Barbut et al., 2005; Owens et al., 2000). In addition, the use of PSE meat affects the sensory quality and has decreased protein functionality in further processed products (Droval et al., 2012; Petracci et al., 2009). PSE meat tissue has an undesirable pale color, soft texture, and surface moisture that leads to an unacceptable appearance during retail display and a dry and tough product after cooking (Schilling, 2009; Van Laack et al., 2000). PSE meat is less acceptable to consumers, has a shorter shelf-life, and lower product yields (Adzitey & Nurul, 2011). Several researchers have examined the reason for the pale appearance in PSE meat (Swatland, 2004, 2008; Warriss, 2000). For example, Warriss (2000) suggested that PSE meat appears pale due to differences in the refractive indices of the sarcoplasm and the myofibrils. In addition, Swatland (2008) proposed that the myofibrils are birefringent in nature and their birefringence is inversely proportional to the pH. Thus, the PSE meat appears paler due to greater light scattering in comparison to normal meat.

PSE meat is differentiated/characterized from normal meat by measuring lightness (CIE $L^*$ values) and pH (Barbut, 1996; Owens et al., 2000; Zhang & Barbut, 2005). Breast meat with $L^*$ values greater than 65 are extremely pale and coupled with low pH values (low final pH and/or rapid pH decline postmortem) and high drip or purge loss percentages are extreme cases of PSE meat (Sams, 1999b). For PSE meat, water holding capacity is significantly impacted due to exudative loss and the loss of water is 1.3 to 7 fold in PSE meat when compared to normal meat at 24 h postmortem (McKee & Sams, 1997; Owens et al., 2000). Lower water holding capacity in PSE meat affects the
processing yield and its use in further processed products (Remignon, Molette, Eadmusik, & Fernandez, 2007). Research has shown that $L^*$ values in poultry breast muscles is affected by various factors such as environmental season, diet, flocks, bird type, feed withdrawal, stunning method, etc. (Smith & Northcutt, 2009). Alvarado & Sams (2003) characterized broiler breast fillets as normal ($L^* < 51$, $pH > 6.2$), and PSE ($L^* > 54$, $pH \leq 6.0$) at 2 h postmortem. The aforementioned studies thus indicate that different $L^*$ and pH values are used as threshold values to identify PSE meat in the poultry industry. Higher $L^*$ values are associated with lower water holding capacity and lower pH (Barbut, 1996; McCurdy et al., 1996; McKee & Sams, 1997, 1998; Rathgeber et al., 1999). Kralik, Djurkin, Kralik, Skrtic, & Radisic (2014) classified broiler breast meat based on the following $L^*$ color values: normal ($L^* 44-53$); PSE ($L^* > 53$); DFD ($L^* < 44$). They reported a negative correlation between $L^*$ and ultimate pH values and a positive correlation between $L^*$ and $b^*$ as well as drip loss. Petracci et al., (2004) observed a higher incidence of pale breast meat ($L^* > 56$) in summer months that was associated with lower ultimate pH and poor water holding capacity. Moreover, Sheard, Hughes, & Jaspal, (2012) characterized broiler breast fillets as normal ($L^* 53-57$), PSE ($L^* > 58$), and dark ($L^* < 52$) and reported a strong negative correlation between pH and $L^*$ ($r = -0.82$). Several investigators have demonstrated that pH measured at 24 h is a better indicator of poultry meat quality than pH measured immediately after slaughter (Fraqueza, Cardoso, Ferreira, & Barreto, 2006; Petracci et al., 2004; Wilkins, Brown, Phillips, & Warriss, 2000). Thus, both $L^*$ (lightness) and pH measurements can be used in determining PSE in poultry meat.
2.6  Poultry meat proteins and functionality

Poultry meat contains approximately 20 to 23% protein (Smith, 2001). Myofibrillar (salt soluble) and sarcoplasmic (water soluble) proteins are the two major protein categories responsible for imparting functionality in poultry products. Protein functionality is measured in terms of water-binding, emulsification, gel formation, adhesion, fat binding, tenderness, texture, and juiciness (Briskey & Kaufman, 1978; Xiong, 1994). Myofibrillar proteins make up 50-55% of the total meat proteins. Myosin consists of 45-55% and actin consists of 20-25% of the myofibrillar proteins, respectively (Pearson & Young, 1989; Smith, 2001). Interaction of myofibrillar proteins (actin and myosin) plays a major role in functional properties of meat (Morrissey, Mulvihill, & O’Neill, 1987; Pearson & Young, 1989; Samejima, Yamauchi, Asghar, & Yasui, 1984; Wang & Smith, 1994). Rigor mortis (stiffening of muscle), occurs postmortem due to the interaction of actin with myosin which results in the formation of permanent actomyosin bonds. Phosphates are used in formed poultry products to dissociate actomyosin bonds into actin and myosin (Smith, 2001), since myosin and actin are more functional in processed products than actomyosin. Sarcoplasmic proteins (glycolytic enzymes, hemoglobin, and myoglobin) make up 30-35% of the total muscle protein and are found in the sarcoplasm (Miller, 1994; Smith, 2001). Proteins are arranged in a three dimensional arrangement, and are denatured by exposure to pH extremes and elevated temperatures during processing (Lehninger, 1982a, 1982b). Factors that influence poultry meat protein functionality and color are rate of pH decline in postmortem muscle and the rate of postmortem carcass temperature decline (Fernandez, Forslid, & Tornberg, 1994; Offer, 1991; Wang & Smith, 1994; Xiong, 1994). Protein properties in processed meat
products are affected by the extent of rigor, pH, mechanical pre-treatment, presence of salts, and temperature (Bailey & Light, 1989; Smith, 2001). Protein-protein and protein-water interaction is important in processed poultry products such as restructured meats, section/formed products, vacuum tumbled, and injected products (Smith, 2001). Some ingredients used to improve protein functionality are salt, phosphates, binders and extenders (collagen, soy protein concentrate, carrageenan, soy protein isolates and flour, whey proteins, etc.) (Daigle, 2005).

There are several implications when PSE meat is used in further processed poultry products. For example, use of PSE turkey meat in the production of turkey breast rolls resulted in poor color uniformity and textural cracking (Ferket, 1995; Owens et al., 2009). Barbut et al., (2005) examined the effect of pale, normal, and dark chicken breast meat on the microstructure, protein extraction, and cooking of marinated fillets. Results suggested that PSE meat had less salt soluble protein extraction and larger intracellular spaces between muscle fibers and bundles in comparison to normal and dark colored meat. Bianchi, Fletcher, & Smith (2005) studied the physical and functional properties of intact and ground pale broiler breast meat. Results indicated higher $L^*$, lower ultimate pH, higher allo-kramer, higher expressible moisture, lower moisture uptake, and higher cook loss in pale fillets when compared to normal fillets.

2.7 Strategies employed to reduce the impact of PSE meat

Numerous strategies have been applied to reduce the impact that PSE meat has on the poultry industry. Some strategies include sorting PSE meat based on $L^*$ and pH values, use of non-meat ingredients such as starch and carrageenan, addition of binders and extenders in further processed products, mixing a known amount of PSE meat with
normal meat, and use of special processing equipment including injectors, tumblers, and massagers (Barbut, 2009). Moreover, some other factors proposed to reduce the incidence of PSE meat include reducing stress during pre-slaughter, reducing transport distance, improving catching conditions, and modification of diet (Petracci et al., 2009). Olivo, Scares, Ida, & Shimokomaki (2001) indicated that dietary supplementation of chicken feed with Vitamin E improved water holding capacity and reduced the incidence of PSE. Marination injection strategies have been employed to improve the functional properties of PSE meat (Alvarado & McKee, 2007; Alvarado & Sams, 2003; Gorsuch & Alvarado, 2010). For example, Alvarado & Sams (2003) investigated the use of high pH phosphate marinades (0.54% NaCl + 0.42% PO₄ (pH 9), 0.54% NaCl + 0.42% PO₄ (pH 11)) in pre-rigor PSE meat. Results showed that these treatments reduced the negative effects of the PSE meat. In addition, Gorsuch & Alvarado (2010) reported that the use of sodium tripolyphosphate, a high pH phosphate (11.9), a high pH sodium tripolyphosphate, an agglomerated phosphate, and a non-agglomerated phosphate improved the color and water holding capacity of pale broiler breast fillets. On the contrary, Woelfel & Sams (2001) indicated that salt and sodium tripolyphosphate marination of post rigor pale broiler breast fillets did not improve the quality of PSE meat. This may be due to differences in the severity of PSE meat. Non-meat ingredients such as enzymes (transglutaminase), starches (potato and tapioca), proteins (whey, soy), and gums (carrageenan's) have been used to improve the functional properties of PSE meat in further processed products (Barbut, 2009). Zhang & Barbut (2005) studied the effects of using regular and modified starches (potato and tapioca) on normal, PSE, and DFD breast meat batters. Results indicated that regular potato starch, modified potato starch, and
regular tapioca starch reduced cooking loss by 2 fold and improved meat protein functionality. Whole muscle proteome of normal and PSE broiler breast meat have not been characterized yet. Proteomics can be applied to identify the potential protein biomarkers responsible for the meat quality differences in normal and PSE broiler breast meat.

2.8 Proteomics methodology

Wilkins et al., (1996) coined the terms proteomics and proteome. Proteomics is defined as the study of the proteome whereas the proteome is the protein complement of the genome at a specific time point. In addition, proteomics has also been defined as “biochemistry at an unprecedented high throughput scale” and “panoramic protein characterization” (Bendixen, 2005). Proteomics is applied to study protein localization, protein-protein interactions, posttranslational modifications, and differential protein expression (Falk, Ramström, Ståhl, & Hober, 2007). Since the 1970’s, two-dimensional electrophoresis (2DE) has been one of the most common methods used to characterize cellular protein expression patterns (O’Farrell, 1975). In addition, 2DE allows the separation of 500-2000 individual proteins from biopsies, tissues and cell cultures (Bendixen, 2005; Görg et al., 2004). 2DE techniques have evolved over the last 30 years with recent improvements in immobilized pH gradients (IPG) based systems (Görg et al., 2000). In one dimensional SDS-PAGE, proteins are solely separated based on their molecular weight whereas in the case of two dimensional gel electrophoresis (2DE), proteins are first horizontally separated based on their isoelectric points (pI’s) using isoelectric focusing (IEF) and then vertically resolved based on their molecular weight (Bendixen, 2005). Protein spots are visualized on the gels using different staining
methods such as coomassie, silver, and fluorescence methods (Rabilloud, Strub, Luche, Van Dorsselaer, & Lunardi, 2001; Rabilloud, 1999, 2000). 2DE with IPG is most often combined with Mass Spectrometry (MS)-based methods to identify the proteins. 2DE with IPG strips has several advantages such as higher resolution, improved reproducibility, and higher loading capacity (Görg et al., 2000). In meat science research, proteomic analysis includes an experiment with muscles of different meat quality traits, protein extraction, IEF, SDS-PAGE 2DE, image and statistical analysis, extraction of significantly changed protein spots, and identification of proteins by MS analysis (Hollung, Veiseth, Jia, Færgestad, & Hildrum, 2007).

2DE-MS protocols includes sample preparation and protein solubilization, protein separation by 2-DE, protein detection and quantitation, computer assisted analysis of 2-DE patterns, protein identification and characterization, and 2-DE protein database construction (Dunn, 1992). In the sample preparation step, proteins are denatured, disaggregated, reduced, and solubilized to disrupt molecular interactions (Görg et al., 2004). Protein solubilization is carried out in a rehydration buffer containing urea, thiourea, 3-3-cholamidopropyldimethylammonio-1-propanesulfonate (CHAPS), Dithiothreitol (DTT), and carrier ampholytes (Görg et al., 2004). A chaotrope such as urea (5-7 M) is used in combination with thiourea (2 M) to denature and disrupt the intra and inter molecular interactions in proteins (Görg et al., 2004). A detergent such as CHAPS (0.5-4%) is used to inhibit hydrophobic interactions between the proteins (Görg et al., 2004). DTT is added at up to 100 mM as a reducing agent for reduction and prevention of re-oxidation of disulfide bonds (Görg et al., 2004). IPG strips are rehydrated with either direct sample loading where the protein sample is included in the
rehydration buffer or a cup loading method where the sample is included in cups following rehydration. After rehydration, IEF focusing is carried out with IPG strips with a pH range from 3-12. IPG strips consist of acrylamide derivatives forming a series of buffers with pK values between 1 and 13 (Blomberg et al., 1995; Corbett, Dunn, Posch, & Görg, 1994). In IEF, proteins are separated based on their respective isoelectric points (pI). After IEF, equilibration of IPG strips is done, where the IPG strips are incubated in equilibration buffers for 10-15 min with gentle shaking. Recommended equilibration buffer composition is 50 mM Tris-HCl (pH 8.8), 2% w/v SDS, 1% w/v DTT, 6 M urea, 4% iodoacetamide, and 30% w/v glycerol (Görg, Postel, & Gunther, 1988). Equilibration is carried out in two steps. In the first step, DTT is added to reduce the sulfhydryl groups. In the second step, iodoacetamide is added to alkylate the reduced sulfhydryl groups (Görg et al., 1987, 1988). After IEF, the proteins are at their respective pI’s with no net charge. SDS containing equilibration buffers are then used to interact with the proteins to make them negatively charged. In the second dimension of SDS-PAGE, the negatively charged proteins are separated based on their molecular weight on 7-15% acrylamide gels. Protein detection on the SDS-PAGE gels is carried out using different staining solutions. The most common staining solutions used for visualization of proteins are coomassie, silver, and fluorescence methods (Rabilloud et al., 2001; Rabilloud, 2000; Rabilloud, 1999). Protein visualization methods are selected based on their sensitivity, linear dynamic range, reproducibility, and their compatibility with MS (Görg et al., 2004). Gel images are captured and subjected to computerized 2-D gel image analysis. Gel image analysis includes: preprocessing of the gel images (image normalization, cropping and background subtraction), spot segmentation, detection and expression
quantification, land marking, spot matching, identification of differentially expressed spots, and data presentation and interpretation (Dowsey, Dunn, & Yang, 2003; Dunn, 1992; Garrels, 1989). In 2DE gel image analysis, two gels (Control vs. treatment) are compared to determine the qualitative and quantitative protein differences (Dowsey et al., 2003).

The final step in proteomic analysis is the identification of proteins using different MS based methods. Prior to subjecting the proteins to MS analysis, gel digestion of proteins is carried out using sequence specific trypsin enzymes to form peptides (Guerrera & Kleiner, 2005). Application of MS to peptides and proteins is known as peptide mass fingerprinting (PMF) (Henzel, Stults, & Watanabe, 1989; Henzel, Watanabe, & Stults, 2003). MS analysis separates the proteins based on their mass to charge ratio (m/z) (Cañas, López-Ferrer, Ramos-Fernández, Camafeita, & Calvo, 2006). Mass spectrometers consist of the following major components: an ionization source, a mass analyzer, an ion detector, data processor, and vacuum pumps (Aebersold & Mann, 2003; Cañas et al., 2006; Guerrera & Kleiner, 2005). In the 1980’s, electrospray ionization (ESI) and Matrix-assisted laser desorption/ionization (MALDI) were used for ionization of peptides and proteins. ESI and MALDI are called soft ionization techniques because they cause little or no fragmentation during the ionization and desorption process (Cañas et al., 2006; Guerrera & Kleiner, 2005). The ESI method ionizes directly from the solution. High voltage is applied to a solvent containing analyte, which flows through a narrow capillary, forming a spray of ions with multiple charged droplets (<10 µm in diameter) at atmospheric pressure (Aebersold & Mann, 2003; Cañas et al., 2006; Guerrera & Kleiner, 2005). For MALDI-MS, protein samples are mixed in appropriate
solvents and co-crystallized with an organic matrix on a metal plate. Sinapinic, α-cyano-4-hydroxycinnamic acids, and 2, 5-dihydroxybenzoic acids are the most common matrixes used for protein and peptide analysis (Billeci & Stults, 1993). A pulsed laser directed at the matrix creates thermal energy to convert the analyte molecules into the gaseous phase (Aebersold & Mann, 2003; Guerrera & Kleiner, 2005). The MALDI ionization technique can tolerate low concentrations of impurities such as buffers and salts in the sample (Cañas et al., 2006; Guerrera & Kleiner, 2005). In MS analysis, several different types of mass analyzers are used, where the ions are separated based on their mass to charge (m/z) ratio. The most common mass analyzers employed in MS analysis are ion trap, Time-Of-Flight (TOF), quadrupole, and Fourier Transform Ion Cyclotron Resonance (FTICR) analyzers (Guerrera & Kleiner, 2005). Ion trap analyzers (three dimensional and linear) trap the ions for a certain time period and are then transferred to the detector (Cañas et al., 2006). In the ion trap analyzers, ions are subjected to increasing voltage causing the ions to destabilize and eject from the trap to the detector. TOF analyzers include a tube under vacuum where the ions are separated based on their flight time and their velocities are inversely proportional to their m/z values (Bienvenut et al., 2002; Medzihradszky et al., 2000). TOF analyzers are mostly used in conjunction with MALDI ionization source (Cañas et al., 2006). Quadrupole analyzers consist of four parallel rods, where voltage is applied, and only the ions with stable trajectories within this oscillating field reach the detector (Leary & Schmidt, 1996; Steel & Henchman, 1998). FTICR analyzers trap the ions using strong magnetic fields (Aebersold & Mann, 2003). Ions in FTICR analyzers display a circular cyclotron motion,
and their m/z ratio is calculated based on their frequency of motion (Guerrera & Kleiner, 2005).

2.9 Application of proteomics in understanding meat quality

Proteomics is the study of the proteome (i.e. set of proteins) which contains information on gene expression and protein translation (Petracci & Cavani, 2012). Two-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has been applied in meat quality research to understand growth and development, postmortem metabolism, calpains role in tenderness, and WHC (Bendixen, 2005; Görg et al., 2004). In addition, proteomics is applied for elucidating protein modifications such as reversible phosphorylation, oxidation, degradation, and denaturation in meat postmortem (Huang & Lametsch, 2013). Several researchers have also focused on understanding early postmortem protein changes using proteomics (D’Alessandro, Marrocco, Zolla, D’Andrea, & Zolla, 2011; Jia et al., 2007; Lametsch et al., 2011; Promeyrat et al., 2011).

Proteomics is an important tool in determining quality biomarkers that are indicators of meat quality defects. Application of proteomics for investigating meat quality is a relatively new approach, but has been recently used to understand the relationship between the muscle proteome and muscle-to-meat conversion (Jia et al., 2007; Jia, et al., 2006; Morzel et al., 2004; Sayd et al., 2006), tenderness (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006; Lametsch et al., 2003; Laville et al., 2009), color (Joseph, Suman, Rentfrow, Li, & Beach, 2012), and water-holding capacity (WHC) (van de Wiel & Zhang, 2007). During muscle to meat conversion, the muscle proteins are significantly impacted. In pigs, proteomics has been applied to understand postmortem protein degradation/modification, meat quality (PSE, WHC, pH), and meat color, whereas in
poultry it has been applied to understand the role of diet on growth and meat quality (Paredi et al., 2012).

Bouley, Chambon, & Picard (2004) studied the mapping of bovine skeletal muscle using two-dimensional gel electrophoresis and mass spectrometry. They identified 129 protein spots corresponding to metabolism, cell structure, cell defense, and contractile apparatus. Jia et al., (2006) analyzed the changes in enzymes associated with energy metabolism during the early postmortem period in *Longissimus Thoracis* bovine muscle using proteomics. Results indicated that 24 proteins (metabolic and heat shock) changed in samples collected at different postmortem times. Jia, et al., (2006) conducted proteome analysis of two bovine muscle types: *M. longissimus dorsi* and *M. semitendinosus*, and discovered that five proteins (cofilin, lactoylglutathione lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP 27, and HSP20) were affected during early post-mortem storage in both muscles. Similarly, Jia et al., (2007) examined the proteome changes up to 24 h postmortem in bovine *Longissimus Thoracis* muscle. Results demonstrated that 47 protein spots changed during the first 24 h postmortem. Application of 2DE in the study identified approximately 1000 individual protein spots. Lamtesch, Roepstroff, & Bendixen (2002) used proteomics (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) to identify protein degradation in *Longissimus dorsi* pig muscle. Nine different proteins including three structural proteins (actin, myosin heavy chain, and troponin T) and six metabolic proteins (glycogen phosphorylase, creatine kinase, phosphopyruvate hydratase, myokinase, pyruvate kinase, and dihydrolipoamide succinyltransferase) changed during postmortem storage. Lametsch et al., (2003) used proteomics to understand the postmortem changes
in porcine muscle and its relationship to meat tenderness by measuring Warner-Bratzler shear force. Results from the study indicated a correlation between the proteins that were identified (actin fragments, myosin heavy chain, myosin light chain II, and triose phosphate isomerase I fragment) and shear force values. Laville et al., (2009) used proteomics to compare sarcoplasmic protein abundance between two groups of pig muscles (tough and tender) that were selected based on Warner-Bratzler shear force values of cooked pork meat. Hwang, Park, Kim, Cho, & Lee (2005) used proteomic methods to assess postmortem proteolysis in pork longissimus muscle to determine the relationship between $L^*$ value and protein proteolysis. Results indicated that eighteen proteins were highly correlated to $L^*$ value. These proteins included actin, myosin light chain, desmin, troponin T, coflin, hemoglobin, ATP synthase, carbonate dehydratase, triose phosphate isomerase, peroxiredoxin, and heat shock protein. In addition, Sayd et al., (2006) evaluated color variability (light pale vs. normal) in pork semimembranosus muscle and correlated variability in color to differences in sarcoplasmic proteome abundance. Mitochondrial and respiratory enzymes (ATP-ase, succinate dehydrogenase, aldehyde dehydrogenase, glucose regulated protein-58kDa, and NADH dehydrogenase) were over-abundant in normal (dark) muscle, indicating that metabolism in the dark muscle was predominantly oxidative. In contrast, pale (light) muscle demonstrated an over-abundance of glycolytic enzymes such as enolase-1, enolase-3, glycerol 3-phosphate dehydrogenase, creatine kinase, and glutathione transferase. These results indicated that the over-abundance of sarcoplasmic proteins in dark muscle is related to myoglobin denaturation and post-mortem pH decline. Thus, the above mentioned studies indicate the
potential application of proteomic tools (2DE in combination with MS) in understanding meat quality problems.

2.10 Application of proteomics in understanding poultry meat quality

Meat muscle contains approximately 20% protein, which makes proteomic methods useful in understanding the relationship between muscle proteins and meat quality defects (Remignon et al., 2006). With respect to poultry, Corzo, Kidd, Dozier, Shack, & Burgess (2006) reported the effect of dietary amino acid scarcity on the muscle proteome. In addition, researchers in the European Union (Doherty et al., 2004) have used proteomic tools to explain changes in muscle proteomes during the growth of laying hens. Furthermore, Remignon et al., (2006) analyzed the proteomes from fast-glycolyzing and normal glycolyzing turkeys, and suggested that the differences in proteomes were indicative of differences in meat quality. This study highlighted the potential of proteomic methods to understand the biochemical basis for color, water-holding capacity, and texture of broiler meat. Molette, Rémignon, & Babilé (2005) studied the proteomic basis of BUT9 turkeys and reported differences in fast and normal glycolyzing breast muscles and their relationship to meat quality. Researchers used two dimensional electrophoresis (2DE) and isoelectric focusing (IEF) to determine that there were differences in three protein spots between normal and fast glycolyzing birds, and the proteins were identified as myosin heavy chain, actin fragments, and glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Mekchay, Teltatham, Nakasathien, & Pongpaichan (2010) conducted proteomic analysis of Thai native and commercial broiler chicken muscles to determine the relationship between the protein composition and tenderness. Results indicated that glycolytic enzymes such as pyruvate kinase, phosphoglycerate
mutase, and triosephosphate isomerase are related to meat quality. In another study, a proteomic approach was used for the identification of chicken meat that was mechanically recovered and hand deboned. Results suggested that the amount of hemoglobin can be used as a marker to differentiate mechanically recovered chicken meat from deboned chicken meat (Surowiec, Koistinen, Fraser, & Bramley, 2011). Proteomic characterization of the sarcoplasmic proteins in the *pectoralis major* and *supracoracoideus* breast muscles was conducted for two different chicken genotypes, which included Ross 708 commercial broilers and Leghorn chicks, Hyline W-36. Results suggested that glycogen phosphorylase, enolase, elongation factor 1, creatine kinase, fructose-bisphosphate aldolase, and glyceraldehyde 3-phosphate-dehydrogenase were different in the two strains during breast muscle growth (Zapata, Reddish, Miller, Lilburn, & Wick, 2012). Phongpa-Ngan, Grider, Mulligan, Aggrey, & Wicker (2011) studied the proteomic analysis and differential expression in proteins that were extracted from chicken with varying growth rates (slow vs. fast growing) and water-holding capacities (WHC) (low vs. high). Proteins identified from chickens using two dimensional electrophoresis and mass spectrometry included metabolic enzymes (creatine kinase, pyruvate kinase, triosephosphate isomerase, ubiquitin), housekeeping proteins (heat shock protein), and contractile proteins (myosin heavy chain, actin).
CHAPTER III
PROTEOME BASIS OF PALE SOFT AND EXUDATIVE CONDITION IN BROILER BREAST (*PECTROALIS MAJOR*) MUSCLES

3.1 Introduction

A combination of rapid postmortem glycolysis and high temperature (above 35°C) can lead to the production of pale, soft, exudative (PSE) poultry meat (Alvarado & Sams, 2003; Pietrzak et al., 1997). PSE meat is associated with a lighter appearance, a softer texture, lower water holding capacity, excessive yield losses, and formation of soft gels (Alvarado & Sams, 2003; Barbut, Zhang, & Marcone, 2005). The use of PSE meat negatively affects the sensory quality and protein functionality of further processed products (Droval et al., 2012; Petracci et al., 2009). PSE meat has been differentiated/characterized from normal meat by lightness ($L^*$ values) and pH (Zhang & Barbut, 2005). Approximately 5 to 40% of poultry breast that is produced in the United States has PSE characteristics, with a greater incidence in the summer months. The production of PSE-like breast meat results in approximately 200 million dollars of annual revenue loss for the poultry industry (Owens et al., 2009; Petracci & Cavani, 2012).

In the post-genomic era, two-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has been more readily applied in meat quality research (Shishkin et al., 2014). Proteomics has been used to understand the relationship between the muscle proteome and muscle-to-meat conversion (Morzel et al., 2004), tenderness
(Lametsch et al., 2003), color (Joseph et al., 2012), and water holding capacity (WHC) (van de Wiel & Zhang, 2007). In poultry, proteomics has been applied to understand the role of diet on broiler growth and meat quality. However, proteomics has not being utilized to characterize the fundamental basis of PSE in chicken. Therefore, the major focus of this study was to identify the proteome differences between normal and PSE chicken breast meat and to determine their relationship to meat quality. The specific objectives of the current study were: (1) to evaluate the differences in cooking loss and shear force of normal and PSE broiler breast meat; (2) to determine differences in descriptive sensory characteristics and consumer acceptance of normal and PSE broiler breast meat; and (3) to correlate meat quality parameters to the differentially abundant muscle proteome components in normal and PSE broiler breast meat.

3.2 Materials and methods

3.2.1 Broiler processing and sampling

Male Hubbard × Cobb 500 birds (n = 1,050) were raised on ad-libitum feed and water for 8 weeks at the Mississippi State University poultry farm. Prior to harvest, a sample of the broilers were subjected to short-term stress, which resulted in an incidence of approximately 20% PSE breast meat. Short-term stressed birds (n = 900) were hand caught, released to a lighted poultry house at 38 °C for 2 h whereas control birds (n = 150) were hand caught, placed in live haul crates at 21 °C for 2 h, and then slaughtered. Stressed (300 birds per day) and control (50 birds per day) broilers were processed on Day 40, 47, and 54 (n = 3 replications) at the Mississippi State University poultry processing facility according to an approved animal welfare protocol (IACUC 13-140). Whole carcasses from the control and stressed birds were stored separately in metal
containers (173 cm in length, 85 cm in width, and 68.5 cm in depth) in ice water from 15 min to 4 h postmortem to mimic the commercial chilling process, and then hand deboned. After deboning, samples were placed in gallon size Ziploc bags (SC Johnson, Racine, WI) and placed in a walk in cooler (2 to 4 °C) for 24 h.

Normal breast meat samples were characterized by a pH at 24 h postmortem (pH_{24}) of 5.8-6.2 and a CIE L* at 24 h postmortem (CIE L_{24}*) of 45-55, and PSE breast meat samples were characterized by a pH_{24} of 5.4-5.7 and a CIE L_{24}* of 55-65. From normal and PSE broiler whole breast meat (pectoralis major) samples, one of the breast halves was used for cooking loss and shear force evaluation, and the remaining breast halves were used for sensory descriptive analysis and consumer acceptability testing. Samples were vacuum packaged (Prime Source Vacuum, Nylon/PE 3 mil standard barrier pouches, 8 × 12 inch, Kansas, MO, USA) and stored at -20 °C until cooking loss, tenderness, sensory descriptive analysis, and consumer acceptability testing were conducted. For pH, color, cooking loss and shear force analysis, normal (18 samples per replication) and PSE broiler (18 samples per replication) breast meat samples were utilized. For proteomic analysis, 5 g samples were cut from a subset of 18 normal (n = 6, 3 samples × 2 replications) and 18 PSE breast fillets (n = 6, 3 samples × 2 replications) and vacuum packaged (Prime Source Vacuum, Nylon/PE 3 mil standard barrier pouches, 6 × 8 inch, Kansas, MO, USA) prior to storage at -80 °C.

3.2.2 pH measurement and Instrumental color evaluation

The pH and color of normal and PSE broiler breast meat samples were determined at the cranial, medial surface (bone side) of the breast muscle at three different locations at 24 h postmortem. The pH_{24} was measured using a pH meter (Model
Accumet 61, Fisher Scientific, Hampton, NH, USA) with an attached meat penetrating probe (Penetration tip, Cole Palmer, Vernon Hills, IL, USA). Color was evaluated using a chroma meter (Model CR-400, Minolta Camera Co Ltd, Osaka, Japan) with an 8 mm port size, 2° observer, and illuminant D65, and expressed as CIE $L^*_{24}$ (lightness), $a^*_{24}$ (redness), and $b^*_{24}$ (yellowness) (Kin et al., 2009).

### 3.2.3 Cooking loss and Warner Bratzler shear force determination

Frozen normal and PSE broiler breast meat samples were thawed overnight at 4 °C for cooking loss and shear force determinations. Samples were individually weighed and baked in a preheated oven (Viking, Greenwood, MS, USA) to a final internal temperature of 77 °C. Internal temperature of the chicken samples was monitored using thermocouples and a data logger (Model UWTR, Omega Engineering, Stamford, Connecticut, USA). After cooking, the chicken samples (normal and PSE) were cooled to room temperature (22 ± 2 °C). Cooking loss of normal and PSE samples was reported as a percentage and calculated as: (initial weight–final weight)/(initial weight) x 100 (Kin et al., 2009). For shear force analysis, six adjacent 1 cm (width) x 1 cm (thickness) x 2 cm (length) strips were cut from each cooked normal and PSE breasts, parallel to the direction of the muscle fibers. Samples were sheared perpendicular to the muscle fibers using a Warner-Bratzler shear attachment that was mounted to an Instron Universal Testing Center (Model 3300, Instron, Norwood, MA, USA) (Meek et al., 2000).

### 3.2.4 Descriptive and Consumer Sensory Analysis

Descriptive sensory analysis of normal and PSE broiler breast meat samples was conducted (trained panelists = 8, replications = 3). Trained panelists had more than 100 h
experience in muscle food sensory evaluation and were trained for 20 h for descriptive analysis of chicken samples according to Meilgaard et al., (2007). During training, the panelists characterized the chicken breast samples with the following sensory descriptive attributes: oral texture (tenderness, juiciness, and overall juiciness), flavor (brothy, chickeny/meaty, rancid, cardboardy, metallic, and off-flavor), and basic tastes (sour, salty, bitter) that have been used as sensory descriptors in previous studies (Fanatico et al., 2007; Lyon, Smith, Lyon, & Savage, 2004; Zhuang, Savage, Kays, & Himmelsbach, 2007; Zhuang & Savage, 2010). Normal and PSE chicken breast samples were cooked as described in the cooking loss section. After cooking, samples were cut into 2.5 cm x 2.5 cm x 2.5 cm cubes, and individually placed in plastic cups that were labeled with three-digit random numbers. Panelists were randomly assigned 4 chicken breast pieces (two pieces each of normal and PSE breast meat samples) that they evaluated on a 0 (none) to 15 (high intensity or extremely) cm line scale on a computer equipped with Compusense version 5.2 (Guelph, Ontario, Canada).

Consumer acceptability testing of normal and PSE chicken breast samples was conducted (panelists = 55 to 60 per replication; replications = 2). Normal and PSE chicken breast samples were cooked as described in the cooking loss section. Cooked breast samples were cooled at room temperature for 15 min, cut into 2.5 × 2.5 × 2.5 cm cubes, and kept warm (60 to 70 °C) in 7.6 L chafer dishes (53042, Polar ware, Kiel, WI) until panelists evaluated the samples but no longer than 30 min. Each panelist received one 2.5 × 2.5 × 2.5 cm piece of both normal and PSE cooked chicken breast in a random order in 57 ml plastic containers that were labeled with three-digit random numbers. Panelists were asked to evaluate the chicken breast sample’s overall acceptability, and the
samples acceptability with respect to appearance, aroma, texture, and flavor on a 9-point hedonic scale on a computer equipped with Compusense version 5.2. The hedonic scale was defined as follows: 9-Like extremely; 8-Like very much; 7-Like moderately; 6-Like slightly; 5-Neither like nor dislike; 4-Dislike slightly; 3-Dislike moderately; 2-Dislike very much; 1-Dislike extremely (Meilgaard et al., 2007). For descriptive and consumer sensory analysis, each panelist was provided with a tray containing coded chicken breast samples, water, apple juice, unsalted crackers, and an expectoration cup.

3.2.5 Whole muscle proteome isolation

Whole muscle proteome of normal and PSE chicken breast samples was extracted according to Lametsch et al., (2003). Frozen normal and PSE muscle tissues (2 g) were homogenized in 8 ml of rehydration buffer (7 M urea, 2 M thiourea, 3% CHAPS, 60 mM DTT, 0.3% Bio-Lyte 3/10 ampholyte (Bio-Rad, Hercules, CA, USA), and 0.001% Bromophenol blue). Homogenates were incubated on ice for 2 h using an orbital shaker (Bellco-Glass Inc, NJ, USA). Subsequently, the homogenate was centrifuged (Sorvall-RC-5C centrifuge, Connecticut, USA) at 10,000 × g for 30 min at 4 °C. Supernatants were collected as the whole muscle proteome.

3.2.6 Two Dimensional Electrophoresis (2-DE)

Protein concentration for each whole muscle proteome extract was determined using the Bradford assay (Bio-Rad). After protein quantification, protein cleanup (~325 µg) was conducted using a cleanup kit (Bio-Rad) prior to suspending the cleaned pellet in 225 µl of rehydration buffer for 30 min at 20 °C. Cleaned protein samples were applied onto immobilized pH gradient (IPG) strips (pH 3-10, 11 cm) and subjected to passive
rehydration for 13 h. After rehydration, first-dimension isoelectric focusing (IEF) was conducted using a Protean IEF system (Bio-Rad). The voltage was initially set at 250V, with a rapid increase in voltage to 8000 V, to achieve a final total voltage of 25 kVh. Subsequently, the IPG strips were equilibrated twice for 15 min each in 4 ml of Equilibration buffer 1 (6 M urea, 30% glycerol, 2.0% SDS, 50 mM Tris HCl, 2.0% DTT, and 0.001% Bromophenol blue) and 4 ml of Equilibration buffer 2 (6 M urea, 30% glycerol, 2.0% SDS, 50 mM Tris HCl, 2.5% iodoacetamide, and 0.001% Bromophenol blue). Proteins were resolved in the second dimension on 12% SDS-PAGE (37.5:1 ratio of acrylamide to bis-acrylamide) using a Criterion system (Bio-Rad). For whole proteome analysis, normal (n = 6, 3 samples × 2 replications) and PSE (n = 6, 3 samples × 2 replications) chicken breast meat samples were used giving a total of 24 gels (2 treatments × 6 birds × duplicate gels). Gels were stained in Coomassie blue (Gelcode, IL, USA) for 24 h and destained until the background stain was removed.

3.2.7 Gel image analysis

Gel images were captured using an imaging system (FOTO Analyst Luminary FX workstation, FotoDyne, Hartland, WI, USA) prior to analysis by PDQUEST software (Bio-Rad). Protein spots from the normal and PSE meat gel images were detected, matched with the aid of landmarks, and normalized by expressing the relative quantity of each spot (ppm) as the ratio of individual spot quantity to the total quantity of each valid spot. Protein spots were considered differentially abundant between normal and PSE groups when they exhibited a 1.5-fold or more intensity difference that was associated with a 5% statistical significance ($P < 0.05$) in the Student’s t-test (Joseph et al., 2012).
3.2.8 Protein identification by mass spectrometry

The excision, reduction, alkylation and in-gel tryptic digestion of protein gel spots were carried out according to Desai et al., (2014). Extracted peptides were concentrated and injected for nano-LC-MS/MS analysis. LC-MS/MS analysis was performed using an LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled with an Eksigent Nanoflex cHiPLC™ system (Eksigent, Dublin, CA) through a nano-electrospray ionization source. Mass analysis method consisted of one segment with eight scan events. First scan event was an Orbitrap MS scan (100–1600 m/z) with 60,000 resolutions for parent ions followed by data dependent MS/MS for fragmentation of the 7 most intense ions with the collision induced dissociation (CID) method. The LC-MS/MS data were submitted to a local MASCOT server for MS/MS protein identification via Proteome Discoverer version 1.3 (Thermo Fisher Scientific) using a custom database of Gallus gallus (chicken) downloaded from Uniprot.

3.2.9 Statistical Analysis

Prior to harvest, a sample of the broilers were subjected to short-term stress (38 °C for 2 h) while the remaining broilers were subjected to control conditions (21 °C for 2 h). From the stressed and control broilers, normal and PSE breast (pectoralis major) meat samples were selected based on pH and color at 24 h postmortem. A randomized complete block design (replications as blocks) with three replications were utilized to test the treatment (normal vs. PSE) effects ($P < 0.05$) on cooking loss, shear force, descriptive sensory analysis, and consumer sensory acceptability (SAS version 9.4, NC, USA). The Fisher’s Protected Least Significant Difference (LSD) test was used to separate the treatment means ($P < 0.05$). For consumer sensory analysis, agglomerative hierarchical
clustering using Wards Method (XLSTAT, New York, USA) was performed to group panelists in clusters based on their liking of broiler breast meat samples. The number of clusters used to group panelists was determined based on a dendrogram and a dissimilarity plot. A randomized complete block design (panelists as blocks) was used within each cluster, and the Fisher’s Protected LSD test was used to separate treatment means within each cluster ($P < 0.05$). In addition, the PROC CORR procedure was used to determine the Pearson’s correlation coefficients between the differentially abundant protein spots and the meat quality parameters ($\text{pH}_{24}$, color ($L^*_{24}$, $a^*_{24}$, $b^*_{24}$), and shear force).

3.3 Results and Discussion

3.3.1 Cooking loss and Warner Bratzler shear force determination

No differences existed ($P > 0.05$) in cooking loss between normal and PSE chicken breast meat samples (Table 3.1). This was unexpected since lower pH in PSE breast meat is usually associated with greater cooking loss, In previous studies, higher cooking loss was reported in PSE (26.2-26.4%) when compared to the cooking loss of normal (21.0-23.0%) broiler breast fillets (Woelfel et al., 2002; Woelfel & Sams, 2001). In the aforementioned studies, normal and PSE breast meat samples were collected from a commercial processing plant and were characterized based on $L^*$, pH, and expressible moisture at 24 h postmortem. However, in the current study, normal and PSE breast fillets were collected from control and stressed birds, and were characterized only based on pH and $L^*$ values measured at 24 h postmortem. In the present study, the lack of difference in cooking loss between normal and PSE breast meat may have been due to
purge loss in the PSE meat due to packaging and freezing. This would minimize differences in cooking loss since the moisture content would be lower in PSE meat.

Shear force values (N) were greater \((P < 0.05)\) for cooked PSE chicken breast meat samples than for normal cooked chicken breast meat samples (Table 3.1). For normal chicken breast strips, 31 out of 324 (10\%) of the strips that were sheared had shear force values greater than 30 N, indicating that these samples were neither tender nor tough. On the other hand, 81 out of 318 (25\%) of the PSE strips that were sheared had shear force values greater than 30 N. In addition, 6 out of 49 (12\%) of the PSE breast samples had strips with shear force values greater than 40 N, indicating that these samples were slightly tough. Previous studies have also demonstrated higher shear force values in PSE broiler breast meat when compared to normal breast meat. Zhuang and Savage (2010) reported Warner-Bratzler shear force values of 47.0 ± 14.7 N and 31.4 ± 15.7 N in light \((L^* > 60)\) and medium \((L^* < 55)\) cooked broiler breast fillets, respectively. Furthermore, Droval et al., (2012) reported shear force values of 24.7 ± 5.7 N and 40.8 ± 11.2 N in normal and PSE cooked broiler breast meat, respectively.
Table 3.1  The cooking loss and shear force of normal and PSE chicken breast meat samples

<table>
<thead>
<tr>
<th>Item</th>
<th>Cooking Loss (%)</th>
<th>Shear Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>20.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PSE</td>
<td>20.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S.E.M</td>
<td>0.28</td>
<td>0.54</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Means with the same letter within a column are not different (P > 0.05).

3.3.2  Descriptive and Consumer Sensory Analysis

Normal breast meat was more tender and juicy (P < 0.05) than cooked PSE breast meat samples (Table 3.2). Descriptive sensory texture scores for normal and PSE cooked chicken breast samples are in agreement with Warner-Bratzler shear force values in which higher shear force values were observed in cooked PSE breast meat when compared to normal breast meat (Table 3.1, Table 3.2). Zhuang and Savage (2010) reported higher intensity scores for textural attributes such as cohesiveness, hardness, rate of breakdown, and chewiness in light (L* > 60) colored broiler breast fillets. PSE breast meat had slightly higher (P < 0.05) cardboardy flavor than normal breast meat, but no other differences (P > 0.05) existed in flavor attributes and basic tastes (Table 3.2). Previous studies have also indicated no differences in the sensory flavor profiles and basic taste attributes of cooked chicken fillets. Fanatico et al., (2007) studied the sensory attributes of slow and fast growing chicken genotypes that were either raised indoors or with outdoor access and reported no differences in most of the basic taste attributes between genotype or between raising method. Moreover, Zhuang and Savage (2010) reported no differences in the flavor intensity scores between light (L* > 60), medium (55 < L* < 60), and dark (L* < 55) broiler breast fillets after cooking.
On average, no differences ($P > 0.05$) were observed in appearance, aroma, flavor, texture and overall acceptability of normal and PSE broiler breast meat samples (Table 3.3). The average scores for appearance, aroma, flavor, texture, and overall acceptability for normal and PSE chicken breast samples ranged between ‘like slightly’ and ‘like moderately’ (Table 3.3). The lack of difference in the consumer acceptability scores between the normal and PSE chicken breast samples may be due to only a subset of panelists differing in their acceptability ratings. To further elucidate the consumers liking of normal and PSE chicken breast meat, agglomerative hierarchical clustering (AHC) was conducted and grouped the panelists into five clusters based on their acceptability ratings for normal and PSE chicken breast meat samples (Table 3.4). Cluster 1 (29.0% of panelists) rated breast meat between ‘like slightly’ and ‘like very much’, and preferred ($P < 0.05$) PSE breast meat over normal. This may be because these consumers like meat that is slightly tender but not too tender or mushy. Cluster 2 (29.9% of panelists) rated the samples between ‘like moderately’ and ‘like very much’, but preferred ($P < 0.05$) normal chicken breast meat over PSE chicken breast meat (Table 3.4). These panelists may have liked these samples more because they prefer chicken to be as tender as possible. Panelists in cluster 3 (15.4%) did not like breast samples from either treatment. Panelists in cluster 4 (3.4%) preferred ($P < 0.05$) PSE chicken breast meat over normal breast meat samples but only consisted of 4 out of 117 panelists (Table 3.4). Panelists in Cluster 5 (22.2%) preferred ($P < 0.05$) normal chicken breast meat over PSE chicken breast meat samples (Table 3.4). The panelists from this group did not like PSE chicken breast meat, which may likely be due to the meat not being as tender as normal meat. In terms of preference, 52.1% of the panelists preferred normal meat over
PSE breast meat and 32.4% of the panelists preferred PSE over normal. Overall, 81% of the consumers liked normal chicken breast meat (score ≥ 6) and 62% of the consumers liked PSE chicken breast meat (scores ≥ 6).

Table 3.2  Sensory descriptive analysis results for normal and PSE baked chicken breast samples with respect to oral texture, flavor, and basic tastes

<table>
<thead>
<tr>
<th>Sensory Attributes</th>
<th>Normal</th>
<th>PSE</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral texture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tenderness</td>
<td>10.3a</td>
<td>8.7b</td>
<td>0.22</td>
</tr>
<tr>
<td>Initial juiciness</td>
<td>9.0a</td>
<td>8.2b</td>
<td>0.18</td>
</tr>
<tr>
<td>Overall juiciness</td>
<td>7.3a</td>
<td>6.2b</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Flavor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brothy</td>
<td>3.0a</td>
<td>2.9a</td>
<td>0.10</td>
</tr>
<tr>
<td>Chickeny/meaty</td>
<td>6.9a</td>
<td>6.8a</td>
<td>0.16</td>
</tr>
<tr>
<td>Rancid</td>
<td>0.3a</td>
<td>0.4a</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardboardy</td>
<td>1.3b</td>
<td>1.9a</td>
<td>0.10</td>
</tr>
<tr>
<td>Metallic</td>
<td>0.9a</td>
<td>1.0a</td>
<td>0.06</td>
</tr>
<tr>
<td>Off-flavor</td>
<td>0.3a</td>
<td>0.4a</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Basic tastes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour</td>
<td>0.4a</td>
<td>0.7a</td>
<td>0.03</td>
</tr>
<tr>
<td>Salty</td>
<td>0.7a</td>
<td>0.6a</td>
<td>0.03</td>
</tr>
<tr>
<td>Bitter</td>
<td>0.5a</td>
<td>0.5a</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*a-b* Means with the same letter within a row are not different (*P* > 0.05).

Hedonic scale was based on a 15-point line scale (0 = none, 15 = extremely or high intensity).
Table 3.3  Mean consumer acceptability scores of appearance, aroma, texture, flavor, and overall acceptability of normal and PSE broiler breast meat determined by consumer panels (n = 117 panelists)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Appearance acceptability</th>
<th>Aroma acceptability</th>
<th>Flavor acceptability</th>
<th>Texture acceptability</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PSE</td>
<td>6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEM</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Means within a column with the same letter are not different within each treatment (P > 0.05).  
Hedonic scale was based on a 9-point scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely).
Table 3.4  Mean hedonic scores for overall consumer acceptability of normal and PSE broiler breast meat according to different clusters of consumers (n = 117 panelists)

<table>
<thead>
<tr>
<th>Cluster</th>
<th>No of Panelists</th>
<th>Panelists (%)</th>
<th>Normal</th>
<th>PSE</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>29.0</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>29.9</td>
<td>7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>15.4</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.17</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>3.4</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
<td>22.2</td>
<td>6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Means within a row with the same letter are not different within each treatment (P > 0.05)

Hedonic scale was based on a 9-point scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely).
3.3.3 Muscle Proteome analysis

Image analyses of the whole muscle proteome gels revealed fifteen differentially abundant protein spots between normal and PSE breast (*pectoralis major*) muscles (Figure 3.1, Table 3.5). Eleven protein spots were over-abundant \((P < 0.05)\) in PSE samples. These proteins were identified as actin alpha, myosin heavy chain (in 2 different spots), phosphoglycerate kinase, creatine kinase M type (in 2 different spots), beta-enolase, carbonic anhydrase 2, proteasome subunit alpha, pyruvate kinase, and malate dehydrogenase (Table 3.6). Four proteins were over-abundant \((P < 0.05)\) in normal samples and were identified as phosphoglycerate mutase-1, alpha-enolase, ATP-dependent 6-phosphofructokinase, and fructose 1, 6-bisphosphatase (Table 3.6). Both myosin heavy chain (spots 2 and 9) and creatine kinase M type (spots 5 and 6) were observed in two different spots (Table 3.6). In the present study, presence of the same protein in two different spots could be attributed to protein fragmentation or post translational modifications (Hamelin et al., 2007; Jia et al., 2009). Poultry muscle fiber types are classified into three main types: Red (Type I/Slow-twitch), Intermediate (Type IIa), and White (Type IIb/Fast-twitch) (Berri, 2000; Foegeding et al., 1996). Poultry breast muscles with a greater proportion of white fibers, have a greater capacity for anaerobic (glycolytic) metabolism, and are fast-fatiguing and therefore used for brief bursts of activity (Foegeding et al., 1996; George & Berger, 1966). Most of the enzymes that were over-abundant in normal and PSE breast (*pectoralis major*) samples were glycolytic enzymes which may be due to their inherent glycolytic muscle fiber composition (Table 3.6).
Figure 3.1 Whole muscle proteome of normal and PSE chicken breast samples subjected to two-dimensional gel electrophoresis utilizing isoelectric focusing (pH 3 to 10; first dimension), SDS-PAGE (12%, second dimension) and coomassie blue staining.

The spot numbers refers to the corresponding proteins identified by tandem mass spectrometry in Table 3.5.
Table 3.5  Differentially abundant sarcoplasmic and myofibrillar proteins in normal and PSE chicken breast (*pectoralis major*) muscles identified by mass spectrometry

<table>
<thead>
<tr>
<th>Spot</th>
<th>Accession number</th>
<th>Protein</th>
<th>Species</th>
<th>ProtScore/matched peptides</th>
<th>Sequence coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>P68139</td>
<td>Actin, alpha skeletal muscle</td>
<td><em>Gallus gallus</em></td>
<td>2087.33/34</td>
<td>78.25</td>
</tr>
<tr>
<td>2</td>
<td>P13538</td>
<td>Myosin heavy chain, skeletal muscle</td>
<td><em>Gallus gallus</em></td>
<td>17768.68/174</td>
<td>47.71</td>
</tr>
<tr>
<td>3</td>
<td>Q5ZLN1</td>
<td>Phosphoglycerate mutase-1</td>
<td><em>Gallus gallus</em></td>
<td>1341.57/26</td>
<td>85.04</td>
</tr>
<tr>
<td>4</td>
<td>F1NU17</td>
<td>Phosphoglycerate kinase</td>
<td><em>Gallus gallus</em></td>
<td>1581.34/36</td>
<td>75.06</td>
</tr>
<tr>
<td>5</td>
<td>P00565</td>
<td>Creatine kinase M-type</td>
<td><em>Gallus gallus</em></td>
<td>617.76/31</td>
<td>61.42</td>
</tr>
<tr>
<td>6</td>
<td>P00565</td>
<td>Creatine kinase M-type</td>
<td><em>Gallus gallus</em></td>
<td>758.88/28</td>
<td>49.08</td>
</tr>
<tr>
<td>7</td>
<td>P07322</td>
<td>Beta-epsilonase</td>
<td><em>Gallus gallus</em></td>
<td>3877.56/31</td>
<td>58.06</td>
</tr>
<tr>
<td>8</td>
<td>P07630</td>
<td>Carboxic anhydrase 2</td>
<td><em>Gallus gallus</em></td>
<td>15481.44</td>
<td>63.85</td>
</tr>
<tr>
<td>9</td>
<td>P13538</td>
<td>Myosin heavy chain, skeletal muscle</td>
<td><em>Gallus gallus</em></td>
<td>2492.60/68</td>
<td>28.42</td>
</tr>
<tr>
<td>10</td>
<td>F1NCO2</td>
<td>Proteasome subunit alpha type</td>
<td><em>Gallus gallus</em></td>
<td>495.16/12</td>
<td>53.26</td>
</tr>
<tr>
<td>11</td>
<td>P00458</td>
<td>Pyruvate kinase PKM</td>
<td><em>Gallus gallus</em></td>
<td>1439.11/39</td>
<td>79.06</td>
</tr>
<tr>
<td>12</td>
<td>F1NZ78</td>
<td>Alpha-epsilonase</td>
<td><em>Gallus gallus</em></td>
<td>1908.23/14</td>
<td>38.71</td>
</tr>
<tr>
<td>13</td>
<td>E1BVT3</td>
<td>Malate dehydrogenase</td>
<td><em>Gallus gallus</em></td>
<td>882.68/21</td>
<td>76.56</td>
</tr>
<tr>
<td>14</td>
<td>Q90YA3</td>
<td>ATP-dependent 6-</td>
<td><em>Gallus gallus</em></td>
<td>1469.57/29</td>
<td>46.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phosphofructokinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Q918D3</td>
<td>Fructose 1,6-bisphosphatase</td>
<td><em>Gallus gallus</em></td>
<td>679.35/15</td>
<td>56.02</td>
</tr>
</tbody>
</table>

For each spot, parameters related to protein identification are provided, including accession number; species; Protscore and number of matched peptides; sequence coverage of peptides in LC-MSMS.

*Spot number refers to the numbered spots in gel image (Figure 3.1)*
Table 3.6  Functional roles of differentially abundant sarcoplasmic and myofibrillar proteins in normal and PSE chicken breast \textit{(pectoralis major)} identified by liquid chromatography-electrospray ionization-tandem Mass Spectrometry (LC-ESI-MS/MS)

<table>
<thead>
<tr>
<th>Spot</th>
<th>Protein</th>
<th>Functional category</th>
<th>Over-abundant category</th>
<th>Spot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Actin, alpha skeletal muscle</td>
<td>Muscle contraction</td>
<td>PSE</td>
<td>1.52$^{b}$</td>
</tr>
<tr>
<td>2</td>
<td>Myosin heavy chain, skeletal muscle</td>
<td>Muscle contraction</td>
<td>PSE</td>
<td>3.35$^{b}$</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoglycerate mutase-1</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>2.11$^{c}$</td>
</tr>
<tr>
<td>4</td>
<td>Phosphoglycerate kinase</td>
<td>Glycolytic enzyme</td>
<td>PSE</td>
<td>2.53$^{b}$</td>
</tr>
<tr>
<td>5</td>
<td>Creatine kinase M-type</td>
<td>ATP regeneration</td>
<td>PSE</td>
<td>1.83$^{b}$</td>
</tr>
<tr>
<td>6</td>
<td>Creatine kinase M-type</td>
<td>ATP regeneration</td>
<td>PSE</td>
<td>2.03$^{b}$</td>
</tr>
<tr>
<td>7</td>
<td>Beta-enolase</td>
<td>Glycolytic enzyme</td>
<td>PSE</td>
<td>2.90$^{b}$</td>
</tr>
<tr>
<td>8</td>
<td>Carbonic anhydrase 2</td>
<td>Hydration of CO$_2$</td>
<td>PSE</td>
<td>2.56$^{b}$</td>
</tr>
<tr>
<td>9</td>
<td>Myosin heavy chain, skeletal muscle</td>
<td>Muscle contraction</td>
<td>PSE</td>
<td>1.68$^{b}$</td>
</tr>
<tr>
<td>10</td>
<td>Proteasome subunit alpha type</td>
<td>Proteolytic enzyme</td>
<td>PSE</td>
<td>1.60$^{b}$</td>
</tr>
<tr>
<td>11</td>
<td>Pyruvate kinase PKM</td>
<td>Glycolytic enzyme</td>
<td>PSE</td>
<td>1.61$^{b}$</td>
</tr>
<tr>
<td>12</td>
<td>Alpha-enolase</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>1.52$^{c}$</td>
</tr>
<tr>
<td>13</td>
<td>Malate dehydrogenase</td>
<td>Energy metabolism</td>
<td>PSE</td>
<td>1.60$^{b}$</td>
</tr>
<tr>
<td>14</td>
<td>ATP-dependent 6-phosphofructokinase</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>2.70$^{c}$</td>
</tr>
<tr>
<td>15</td>
<td>Fructose 1,6-bisphosphatase (Fragment)</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>1.56$^{c}$</td>
</tr>
</tbody>
</table>

$^{a}$ Spot number refers to the numbered spots in gel image (Figure 3.1).

$^{b}$ Spot ratio of PSE/normal.

$^{c}$ Spot ratio of normal/PSE.
Table 3.7  Pearson's correlation between meat quality attributes (pH, color, and shear force) and differentially abundant sarcoplasmic and myofibrillar proteins in normal and PSE *pectoralis major* muscles

<table>
<thead>
<tr>
<th>Protein</th>
<th>Over-abundant category</th>
<th>pH24</th>
<th>L*24</th>
<th>a*24</th>
<th>b*24</th>
<th>Shear force</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin, alpha skeletal muscle</td>
<td>PSE</td>
<td>-0.65*</td>
<td>0.56</td>
<td>-0.06</td>
<td>0.26</td>
<td>0.73*</td>
</tr>
<tr>
<td>Myosin heavy chain, skeletal muscle</td>
<td>PSE</td>
<td>-0.78*</td>
<td>0.67*</td>
<td>-0.04</td>
<td>0.40</td>
<td>0.66*</td>
</tr>
<tr>
<td>Phosphoglycerate mutase-1</td>
<td>Normal</td>
<td>0.61*</td>
<td>-0.57*</td>
<td>0.39</td>
<td>-0.21</td>
<td>-0.41</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td>PSE</td>
<td>-0.90*</td>
<td>0.87*</td>
<td>-0.07</td>
<td>0.48</td>
<td>0.58</td>
</tr>
<tr>
<td>Creatine kinase M-type</td>
<td>PSE</td>
<td>-0.81*</td>
<td>0.80*</td>
<td>-0.31</td>
<td>0.63</td>
<td>0.88*</td>
</tr>
<tr>
<td>Beta-enolase</td>
<td>PSE</td>
<td>-0.94*</td>
<td>0.93*</td>
<td>-0.29</td>
<td>0.59</td>
<td>0.68*</td>
</tr>
<tr>
<td>Carbonic anhydrase 2</td>
<td>PSE</td>
<td>-0.83*</td>
<td>0.90*</td>
<td>-0.21</td>
<td>0.58</td>
<td>0.70*</td>
</tr>
<tr>
<td>Proteasome subunit alpha type</td>
<td>PSE</td>
<td>-0.66*</td>
<td>0.62</td>
<td>-0.09</td>
<td>0.32</td>
<td>0.70*</td>
</tr>
<tr>
<td>Pyruvate kinase FKM</td>
<td>PSE</td>
<td>-0.65*</td>
<td>0.66*</td>
<td>-0.60</td>
<td>0.73*</td>
<td>0.31</td>
</tr>
<tr>
<td>Alpha-enolase</td>
<td>Normal</td>
<td>0.73*</td>
<td>-0.65*</td>
<td>0.45</td>
<td>-0.40</td>
<td>-0.66*</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>PSE</td>
<td>-0.88*</td>
<td>0.81*</td>
<td>-0.35</td>
<td>0.56</td>
<td>0.53*</td>
</tr>
<tr>
<td>ATP-dependent 6-phosphofructokinase</td>
<td>Normal</td>
<td>0.78*</td>
<td>-0.90*</td>
<td>0.22</td>
<td>-0.76*</td>
<td>-0.34</td>
</tr>
<tr>
<td>Fructose 1,6-bisphosphatase (Fragment)</td>
<td>Normal</td>
<td>0.67*</td>
<td>-0.55</td>
<td>0.35</td>
<td>-0.20</td>
<td>-0.42</td>
</tr>
</tbody>
</table>

*P < 0.05
3.3.3.1 Glycolytic enzymes

3.3.3.1.1 Phosphoglycerate kinase (PGK) and Beta-enolase

Phosphoglycerate kinase (PGK) and beta-enolase were over-abundant \( (P < 0.05) \) in PSE meat (Table 3.6). During glycolysis, PGK produces ATP and 3-phosphoglycerate from ADP and 1,3-bisphosphoglycerate, and enolase converts phosphoglycerate into phosphoenolpyruvate (Keller et al., 2000; Ohlendieck, 2010; Watson & Littlechild, 1990). Jia et al., (2006) studied the changes in enzymes associated with energy metabolism during antemortem and early postmortem in longissimus thoracis bovine muscle and results indicated an over-abundance of phosphoglycerate kinase (PGK) and beta-enolase in longissimus muscles postmortem compared to muscles collected antemortem. These enzymes contributed to an increased rate of glycolysis and meat quality variation in longissimus muscle. In our study, PGK was 2.5 fold and beta-enolase was 2.9 fold over-abundant in PSE meat (Table 3.6) and is indicative of an increased glycolytic activity and hence a lower pH in PSE meat. Furthermore, in the present study, PGK and beta-enolase were negatively correlated with pH\textsubscript{24} and positively with \( L^*\text{24} \) and shear force (Table 3.7).

3.3.3.1.2 Pyruvate kinase M type (PKM)

Pyruvate kinase M type (PKM) was over-abundant \( (P < 0.05) \) in PSE meat (Table 3.6). This is a key enzyme involved in the last step of the glycolytic cycle, which converts phosphoenolpyruvate to pyruvate (Fontanesi et al., 2008). Mekchay et al., (2010) used a proteomic approach to determine the tenderness differences in Thai native and commercial broiler chicken muscles and reported that pyruvate kinase muscle type
(PKM), phosphoglycerate mutase (PGAM), and triose phosphate isomerase (TPI) were 1.3 fold low in abundance in the low-shear force group of Thai native chicken samples. Similarly, in the present study, PKM was 1.6 fold less abundant \( (P < 0.05) \) in normal meat with lower shear force values (Table 3.1, Table 3.6). Lametsch et al., (2011) studied the postmortem changes in pork muscle protein phosphorylation in relation to the RN genotype and indicated that glycogen phosphorylase, phosphofructokinase, and pyruvate kinase (PKM) were affected by the RN genotype. Moreover, the results indicated that the phosphorylation of these enzymes might be related to the increased rate of pH decline in the RN genotype. Similar to phosphoglycerate kinase and beta-enolase, the overabundance of PKM in PSE meat was negatively correlated with pH\(_{24}\) and positively correlated with \(L^*_{24}\) and \(b^*_{24}\) (Table 3.7).

### 3.3.3.1.3 Phosphoglycerate mutase-1 (PGM-1)

Phosphoglycerate mutase-1 (PGM-1) was over-abundant \( (P < 0.05) \) in normal meat (Table 3.6). This enzyme converts 3-phosphoglycerate into 2-phosphoglycerate using 2,3 biphosphoglycerate as a cofactor in the glycolytic cycle (Qiu, Zhao, Xu, Yerle, & Liu, 2008). Proteomic analysis of Thai native and commercial broiler chicken muscles indicated that pyruvate kinase muscle, phosphoglycerate mutase (PGM), and triose phosphate isomerase were 1.3 fold less abundant in the low-shear force group of Thai native chicken samples when compared to the commercial samples (Mekchay et al., 2010). In contrast, in our study, PGM-1 was 2.0 fold over-abundant in normal meat samples when compared to PSE samples (Table 3.6). The differences in the abundance of PGM-1 may be due to genetic variability in the chickens, i.e. commercial broiler chickens in the present study instead of Thai native chickens that were evaluated in the referenced
Moreover, in the present study, PGM-1 over-abundant in normal meat, correlated positively with pH and negatively with \( L^* \) (Table 3.7).

### 3.3.3.1.4 Alpha-enolase

Alpha-enolase was over-abundant \((P < 0.05)\) in normal meat (Table 3.6). Di Luca, Elia, Hamill, & Mullen (2013) examined the proteome profile of pork longissimus muscles with varying levels of drip loss and reported the presence of alpha-enolase in drip. In contrast, in our study, the proteome in normal and PSE meat drip loss was not taken into consideration. It will be interesting to determine if alpha-enolase is greater in the drip loss from PSE breast meat when compared to normal meat. Moreover, in the present study, alpha-enolase was over-abundant in normal meat and was positively correlated with pH and negatively correlated with \( L^* \) and shear force (Table 3.7).

### 3.3.3.1.5 ATP dependent 6-phosphofructokinase (PFK)

ATP dependent 6-phosphofructokinase (PFK) was over-abundant \((P < 0.05)\) in normal meat (Table 3.6). In the glycolytic cycle, PFK is a rate limiting enzyme that converts fructose-6-phosphate to fructose-1,6-biphosphate (Ohlendieck, 2010). A previous study on the relationship between postmortem changes in pork muscle and RN genotype indicated that glycogen phosphorylase, PFK and pyruvate kinase (PKM) were affected by the RN genotype (Lametsch et al., 2011). Moreover, the results indicated that the phosphorylation of these enzymes might be related to the increased rate of pH decline in the RN genotype. In contrast, PFK was over-abundant in normal meat in the present study. The differences in protein abundance may be due to species, since pectoralis major broiler muscles were evaluated in the present study, in comparison to pork
*longissimus* muscles in the referenced study. In the present study, PFK abundance was positively correlated with pH24 and negatively correlated with *L*^*a*^ and *b*^*a*^ (Table 3.7).

### 3.3.3.1.6 Fructose 1, 6-bisphosphatase (FBP)

Fructose 1, 6-bisphosphatase (FBP) was over-abundant (*P* < 0.05) in normal meat (Table 3.6). FBP and aldolase catalyze the hydrolysis of fructose 1,6-bisphosphate to fructose 6-phosphate (Rakus, Pasek, Krotkiewski, & Dzugaj, 2004). Doherty et al., (2004) studied the soluble proteome changes in layered chicken *pectoralis* muscle from 1 to 27 days after hatching. In this study, increase in the birds’ age from day 1 to 27 resulted in a 44 fold increase in *pectoralis* muscle mass, which led to a substantial increase in glycolytic enzymes in the soluble proteome. Fructose 1, 6-bisphosphatase (FBP) was identified as one of the proteins in the soluble *pectoralis* muscle proteome. The current study indicates that glycolytic enzymes (phosphoglycerate mutase-1, alpha-enolase, ATP-dependent 6-phosphofructokinase, and fructose 1, 6-bisphosphatase) were over-abundant in normal meat. Therefore, further research is needed to examine the role of these enzymes in the accelerated postmortem glycolysis that was observed in PSE meat.

### 3.3.3.2 Myofibrillar proteins

Two myofibrillar proteins (actin alpha and myosin heavy chain) that are involved in muscle contraction were over-abundant (*P* < 0.05) in the PSE group (Table 3.6). A myosin molecule consists of two myosin heavy chains, two alkali light chains, and two other light chains (Rathgeber, 2000). Choi, Ryu, & Kim (2007) evaluated the influence of myosin light chain and heavy chain isoforms on the early postmortem glycolytic rate and
meat quality traits in porcine *longissimus* muscle. In this study, the myosin heavy chain isoform was over-abundant in the fast-glycolyzing group when compared to the normal-glycolyzing group. Moreover, in the same study, myosin heavy chain (fast/slow) ratio was negatively correlated to early postmortem muscle pH \( (r = -0.51) \). Similarly, in our study, myosin heavy chain was over-abundant in PSE meat and negatively correlated with pH\textsubscript{24} (Table 3.7). Lametsch et al., (2003) examined the postmortem proteome changes in porcine muscles and related the proteome changes to tenderness. In this study, actin and myosin heavy chain spot intensity was negatively correlated \( (r = -0.44 \text{ to } -0.55) \) whereas myosin light chain spot intensity was positively correlated \( (r = 0.49 \text{ to } 0.59) \) with Warner-Bratzler shear force. In a similar study, postmortem proteolysis and its relationship to meat quality was examined in pig *longissimus* muscle and results indicated that myosin light chain 1 and actin were positively correlated \( (r = 0.70 \text{ to } 0.75) \) with Warner-Bratzler shear force (Hwang et al., 2005). Similar to other studies, actin alpha and myosin heavy chain over-were abundant in PSE meat and positively correlated \( (r = 0.66 \text{ to } 0.73) \) with shear force (Table 3.7).

### 3.3.3.3 Malate dehydrogenase (MDH)

Malate dehydrogenase (MDH) was over-abundant \( (P < 0.05) \) in PSE meat (Table 3.6). This enzyme catalyzes the interconversion of oxaloacetate and malate utilizing the NAD/NADH coenzyme system and also plays an important role in the malate/aspartate shuttle across the mitochondrial membrane (Minárik, Tomaásková, Kollárová, & Antalík, 2002). Previous studies have identified malate dehydrogenase (MDH) as a potential meat quality marker. Laville et al., (2007) compared the sarcoplasmic proteome between two different groups (tough and tender) of *longissimus* pig muscles and fourteen proteins
were differentially abundant between the two groups. MDH was over-abundant in the tough group. Te Pas et al., (2013) identified MDH as a potential marker in pork longissimus dorsi muscles with varying ultimate pH and drip loss. In our study, the over-abundance of MDH in PSE meat was negatively correlated with pH$_{24}$ and positively correlated with $L^*$$_{24}$ and shear force (Table 3.7). Although the present study indicates an over-abundance of MDH in PSE meat, further investigation is needed to elucidate the role of MDH in the accelerated postmortem glycolysis that is observed in PSE meat.

3.3.3.4 Creatine kinase M-type (CKM)

Creatine kinase M type (CKM) was over-abundant in PSE meat (Table 3.6). CKM converts creatine phosphate into creatine and ATP and has both structural and enzymatic functions (Phongpa-Ngan et al., 2011; van de Wiel & Zhang, 2007). Rathgeber et al., (1999) detected two glycolytic enzymes (creatine kinase and glycogen phosphorylase) in the myofibrillar protein extract of rapid glycolyzing turkey breast meat (pH ≤ 5.8 at 15 min postmortem) that were not present in normal glycolyzing (pH > 6.0 at 15 min postmortem) turkey breast meat. In addition, previous research reported that CKM was overabundant in chickens with increased growth rate and lower water-holding capacity (Phongpa-Ngan et al., 2011). Sayd et al., (2006) investigated the sarcoplasmic protein expression in pork semimembranosus muscles that were selected based on $L^*$ values (light and dark) at 36 h postmortem. Similar to our results, CKM was over-abundant in light muscles when compared to their darker counterparts. In addition, Laville et al., (2005) characterized PSE zones in pig semimembranosus muscle and reported that 16 proteins were affected in PSE meat, in particular, myosin light chain, creatine kinase, and troponin T were over-abundant in PSE meat when compared with control meat. Based on
these reports, the over-abundance of CKM could be related to lower (pH$_{24}$) and waterholding capacity, and higher $L^*_{24}$, shear force, and the incidence of PSE meat (Table 3.7).

3.3.3.5 Carbonic anhydrase 2

Carbonic anhydrase 2 was over-abundant in PSE meat (Table 3.6). Carbonic anhydrase 2, also known as carbonate dehydratase, catalyzes the reversible hydration of carbon dioxide (Hamelin et al., 2007). Previous investigations have identified carbonic anhydrase as a possible meat quality marker. Hwang et al., (2005) studied the postmortem proteolysis and its relation to meat quality in in pig *longissimus* muscles and identified carbonic anhydrase as a potential biomarker for determining pork quality. Furthermore, Damon et al., (2013) examined the association between gene expression and meat quality in pork *longissimus* muscle and reported that carbonic anhydrase gene expression was negatively correlated with ultimate pH and positively correlated with drip loss and $L^*$ value. Similar results were found in our study, in which carbonic anhydrase was negatively correlated with pH$_{24}$ and positively correlated with $L^*_{24}$ and shear force (Table 3.7).

3.3.3.6 Proteasome subunit alpha

Proteasome subunit alpha was over-abundant in PSE meat ($P < 0.05$) and is involved in postmortem protein degradation (Sentandreu, Coulis, & Ouali, 2002). Jia et al., (2006) studied the changes in enzymes associated with antemortem and early postmortem energy metabolism in beef *longissimus thoracis* muscle and reported an over-abundance of proteasome subunit alpha in postmortem muscle samples when compared to samples collected antemortem. In a similar study, Jia et al., (2007) examined...
proteome changes in *longissimus thoracis* bovine muscle at different postmortem storage periods (1, 2, 3, 6, 10, and 24 h). Results indicated that abundance of the proteasome subunit beta increased at 24 h postmortem. In the present study, proteasome subunit alpha was over-abundant in PSE meat and was negatively correlated with pH and positively correlated with shear force values (Table 3.7).

### 3.4 Conclusions

The results of the present study indicate that the over-abundance of proteins involved in (*glycolytic, muscle contraction, proteolytic, ATP regeneration, energy metabolism, and CO₂ hydration*) in PSE breast meat may be related to the meat quality differences between normal and PSE breast meat. Based on the protein markers identified in the present study, future studies should focus on identifying the genes responsible for differences in protein abundance and their relationship to poultry meat quality. In addition, further studies will focus on stressing birds of different genetic strains to find out how the protein abundance is related to genetic selection in order to help decrease the incidence of PSE meat in the poultry industry. Since woody breast meat, also described as muscle myopathy, is prevalent in the poultry industry, proteomics could be utilized to understand differences in protein abundance due to muscle myopathy.
CHAPTER IV
PROTEOME BASIS OF PALE, SOFT AND EXUDATIVE CONDITION IN BROILER
BREAST MEAT FROM A COMMERCIAL PROCESSING PLANT

4.1 Introduction

Due to changes in consumer eating and purchasing patterns, there has been a shift in how chicken products are marketed. In the 1960’s, over 80% of chicken broilers were marketed as whole carcasses, and only 2% were sold as further processed products. In contrast, in 2011, 12% of chicken broilers were sold as whole carcasses, 42% as cut up parts, and 46% as further processed products (MacDonald, 2014). Moreover, the increase in demand for cut-up or deboned chicken parts has resulted in meat quality problems that are related to water holding capacity, appearance, and texture (Petracci & Cavani, 2012). Pale, Soft, Exudative (PSE) meat has a lighter appearance, a softer texture, lower water holding capacity, excessive yield losses, and formation of soft gels when compared to normal meat (Alvarado & Sams, 2003; Barbut, Zhang, & Marcone, 2005; Owens, Hirschler, Mckee, Martinez-Dawson, & Sams, 2000). It has been estimated that the production of PSE-like breast meat results in approximately 200 million dollars of annual revenue loss for the poultry industry (Owens, Alvarado, & Sams, 2009; Petracci & Cavani, 2012). Moreover, it has been reported that approximately 5-40% of poultry products in the United States are PSE, which is dependent on the season of the year (Owens et al., 2009).
Lean broiler meat contains approximately 20% protein and 75% water, which makes proteomic methods useful in understanding the relationship between muscle proteins and meat quality (Remignon et al., 2006). Two-dimensional electrophoresis (2-DE) in combination with mass spectrometry (MS) has been applied in meat quality research to understand growth and development, postmortem metabolism, the role of calpains in tenderness, and water holding capacity (Bendixen, 2005; Görg et al., 2004). Proteomics has been applied in poultry research to understand the role of diet on broiler growth and meat quality (Paredi et al., 2012). In addition, researchers have related muscle proteomes to the growth and development of laying hens (Doherty et al., 2004). Furthermore, researchers analyzed the proteomes from fast-glycolyzing and normal glycolyzing turkeys, and suggested that the differences in proteomes were indicative of differences in meat quality (Molette et al., 2005). We have previously demonstrated the use of proteomic methods in understanding the red color meat quality defect in channel catfish (*Ictalurus punctatus*) fillets (Desai et al., 2014). Proteomics has not been utilized to determine protein biomarkers in PSE chicken breast meat from commercial processing plants. Therefore, the specific objectives of the current study were: (1) to characterize the whole muscle proteomes of normal and PSE broiler breast meat from a commercial processing plant; and (2) to evaluate the differences in meat quality traits (pH, color, cooking loss, and shear force) of normal and PSE broiler breast meat from a commercial processing plant.
4.2 Materials and methods

4.2.1 Broiler breast meat sampling

Normal and PSE chicken breast meat (pectoralis major) samples were collected based on visual color from a deboning line in a commercial processing plant on three separate occasions (n = 3 replications). Normal and PSE chicken breast samples were transported for approximately 120 min to Mississippi State University in ice-filled coolers. Normal (12 samples per replication) and PSE (12 samples per replication) chicken breast samples were verified based on pH and instrumental color (n = 3 replications) on the day of sampling. Normal breast meat samples were characterized by pH (5.8-6.2) and CIE L* (45-55), and PSE breast meat samples were characterized by pH (5.4-5.7) and CIE L* (≥ 60). Normal and PSE chicken breast samples were labeled and vacuum packaged separately (Prime Source Vacuum, Nylon/PE 3 mil standard barrier pouches, 8 × 12 inch, Kansas, MO, USA) and stored at -20 °C until cooking loss and tenderness measurements were determined. For proteomic analysis, approximately 5 g samples from the same normal and PSE breast fillets (3 samples per replication) were cut and vacuum packaged separately (Prime Source Vacuum, Nylon/PE 3 mil standard barrier pouches, 6 × 8 inch, Kansas, MO, USA) prior to storage at -80 °C.

4.2.2 pH measurement and Instrumental color evaluation

The ultimate pH (pH24) of normal (12 samples per replication) and PSE broiler (12 samples per replication) breast meat samples was determined using a pH meter (Model Accumet 61, Fisher Scientific, Hampton, NH, USA) with an attached meat penetrating probe (Penetration tip, Cole Palmer, Vernon Hills, IL, USA), by direct insertion into the cranial, medial surface (bone side) of the breast muscle at three
different locations. Instrumental color was determined using a chroma meter (Model CR-400, Minolta Camera Co Ltd Osaka, Japan) with an 8 mm port size, 2° observer, and illuminant D65. Calibration of the instrument was carried out using a standard white Minolta calibration plate (Model No 20933026, Osaka, Japan). Color of normal (12 samples per replication) and PSE (12 samples per replication) breast meat samples at 24 h postmortem was measured and expressed as CIE $L^*_{24}$ (lightness), $a^*_{24}$ (redness), and $b^*_{24}$ (yellowness) (Kin et al., 2009).

### 4.2.3 Cooking loss

Frozen normal (12 samples per replication) and PSE (12 samples per replication) chicken breast samples were thawed overnight at 4 °C. Samples were weighed, placed separately on aluminum foil on a baking sheet, and baked in a preheated oven (Viking, Greenwood, MS, USA) to a final internal temperature of 77 °C. The internal temperature of the chicken samples was monitored using thermocouples and a data logger (Model UWTR, Omega Engineering, Stamford, Connecticut, USA). After cooking, the chicken samples (normal and PSE) were cooled to room temperature (22 ± 2 °C) and residual excess moisture was removed from each chicken sample with a paper towel prior to reweighing. Cooking loss of normal (12 samples per replication) and PSE (12 samples per replication) chicken breast samples was reported as a percentage and calculated as: $(\text{initial weight–final weight})/(\text{initial weight}) \times 100$ (Kin et al., 2009).

### 4.2.4 Warner Bratzler shear force determination

Tenderness of normal (12 samples per replication) and PSE (12 samples per replication) samples was assessed using an objective texture procedure as described in
Meek et al., (2000). Normal and PSE chicken breast samples that were used for cooking loss determinations were cooled to room temperature (22 ± 2 °C) and used for shear force (N) determinations. Six adjacent 1 cm (width) x 1 cm (thickness) x 2 cm (length) strips were cut from each cooked breast, parallel to the direction of the muscle fibers. Samples were sheared perpendicular to the muscle fibers using a Warner-Bratzler shear attachment that was mounted to an Instron Universal Testing Center (Model 3300, Instron, Norwood, MA, USA).

4.2.5 Whole muscle proteome isolation

The whole muscle proteome of normal (3 samples per replication) and PSE (3 samples per replication) chicken breast samples were extracted according to Lametsch et al., (2003). Frozen normal and PSE chicken breast muscle tissues (2 g) were homogenized (FSH 500 homogenizer, Thermo Fisher Scientific, MA, USA) in 8 ml of rehydration buffer (7 M urea, 2 M thiourea, 3% CHAPS, 60 mM DTT, 0.3% Bio-Lyte 3/10 ampholyte (Bio-Rad), and 0.001% Bromophenol blue). The homogenates were incubated on ice for 30 min using an orbital shaker (Bellco Glass Inc, NJ, USA). Subsequently, the homogenate was centrifuged (Sorvall RC-5C Plus Super Speed Centrifuge, Newton, Connecticut, USA) at 10,000 × g for 30 min at 4 °C. Supernatants were collected as the whole muscle proteome.

4.2.6 Two dimensional electrophoresis (2-DE)

The protein concentration of the whole muscle proteome extracts was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). After protein quantification, protein cleanup (~325 µg) was conducted using the Ready Prep™ 2-D Cleanup Kit (Bio-
Rad) prior to suspending the cleaned sample (~325 µg) in 225 µl of rehydration buffer (7 M urea, 2 M thiourea, 3% CHAPS, 60 mM DTT, 0.3% Bio-Lyte 3/10 ampholyte (Bio-Rad), and 0.001% Bromophenol blue) at room temperature for 30 min. After the pellets were solubilized, tubes were centrifuged (Eppendorf Centrifuge 5415C, Brinkmann Instruments, Westbury, NY) at 14,000 × g for 5 min at 20 ºC. Two hundred microliters of the cleaned protein samples were then loaded onto immobilized pH gradient (IPG) strips (pH 3-10, 11cm) and subjected to passive rehydration for 13 h, prior to first-dimension isoelectric focusing (IEF) in a protean IEF cell system (Bio-Rad). The voltage was initially set at 250V, with a rapid increase in voltage to 8000 V, to achieve a final total voltage of 25 kVh. Subsequently, the IPG strips were equilibrated twice for 15 min each in 4 ml of Equilibration buffer 1 (6 M urea, 30% glycerol, 2% SDS, 50 mM Tris- HCl, 2% DTT, and 0.001% Bromophenol blue) and 4 ml of Equilibration buffer 2 (6 M urea, 30% glycerol, 2% SDS, 50 mM Tris-HCl, 2.5% iodoacetamide, and 0.001% Bromophenol blue). The proteins were resolved in the second dimension on 12% SDS-PAGE (37.5:1 ratio of acrylamide to bis-acrylamide) using a Criterion gel electrophoresis system (Bio-Rad). For whole muscle proteome analysis, normal (3 samples per replication) and PSE (3 samples per replication) chicken breast samples were used giving a total of 18 gels (2 treatments × 9 birds). The gels were stained in Coomassie Blue (Gelcode TM, Blue Safe Protein Stain, Rockford, IL, USA) for 24 h and destained until the background stain was removed and the protein spots on the gel surface became clear. All normal and PSE chicken breast meat gels were run under the same conditions.
4.2.7 Gel image analyses

The gel images were captured using an imaging system (FOTO Analyst Luminary FX workstation, FotoDyne, Hartland, WI, USA) prior to analysis by PDQUEST software (Bio-Rad). For comparative gel image analysis, images were divided into two groups. The first group consisted of normal gel images, and the second group consisted of PSE gel images. Protein spots from the normal and PSE meat gel images were detected, matched with the aid of landmarks, and normalized by expressing the relative quantity of each spot (ppm) as the ratio of individual spot quantity to the total quantity of valid spot. Protein spots were considered differentially abundant between normal and PSE groups when they exhibited a 1.5-fold or more intensity difference that was associated with a 5% statistical significance (\(P < 0.05\)) in the Student’s t-test (Joseph et al., 2012).

4.2.8 Protein identification by mass spectrometry

Protein isolation and mass spectrometry analysis were done according to Desai et al., (2014). Protein spots that were differentially abundant between normal and PSE treatments were excised from the gels with pipette tips. The excised protein spots were destained with 50 mM NH\(_4\)HCO\(_3\)/50% CH\(_3\)CN for 30 min, then dried in a vacuum centrifuge. Protein spots were reduced and alkylated in 10 mM DTT and 50 mM iodoacetamide, prior to incubation in the dark for 30 min at room temperature. Gel pieces were then washed with 50 mM NH\(_4\)HCO\(_3\) and 50% CH\(_3\)CN, and vacuum dried. Tryptic digestion was carried out using proteomic grade trypsin (20 ng/\(\mu\)l; Sigma-Aldrich, St. Louis, MO, USA) on ice for 1 h, prior to incubation at 37 °C for 18 h. Peptide extraction was carried out in 50% CH\(_3\)CN/0.1% trifluoroacetic acid. Desalting and concentration of
the peptide extracts was done using a 0.1-10 µl pipette tip (Sarstedt, Newton, NC, USA) packed with 1 mm of Empore C-18 (3M, St. Paul, MN, USA).

Mass spectrometric analysis of peptide mixtures was carried out using a MALDI TOF-TOF Proteomics analyzer (Applied Biosystems, Foster City, CA, USA). Desalted peptide extracts were eluted with 0.3 µl of matrix solution (5 mg/ml α-cyano-4-hydroxycinnamic acid, (Sigma-Aldrich, St. Louis, MO, USA) in 50% CH$_3$CN/0.1% trifluoroacetic acid), and spotted on a MALDI Opti-TOF 384 well insert (Applied Biosystems, Foster City, CA, USA). An initial MALDI-MS spectrum was acquired with about 400 laser shots per spectrum and a maximum of 15 peaks with a signal-to-noise ratio of greater than 20 were automatically selected for MS-MS analysis (1000 shots per spectrum) by post-source decay. MS-MS identification spectra were submitted to a database similarity search using Protein Pilot Version 4.0 (Applied Biosystems, Foster City, CA, USA) with a custom based Uniprot database of chicken (*Gallus gallus*) sequences available from the National Center for Biotechnology Information (NCBI) database.

### 4.2.9 Statistical Analysis

A randomized complete block design (replications as blocks) with three replications was utilized to test the treatment (normal vs. PSE) effects ($P < 0.05$) on pH, color, cooking loss, and shear force. When differences occurred ($P < 0.05$) among treatments, the Fisher’s Protected Least Significant Difference (LSD) test was used to separate the treatment means.
4.3 Results and discussion

4.3.1 pH measurement and Instrumental color evaluation

The average pH\textsubscript{24} values of normal chicken breast meat were greater ($P < 0.05$) than the pH\textsubscript{24} of PSE chicken breast (Table 4.1). Subjecting broilers to short-term or acute stress prior to slaughter can cause a rapid buildup of lactic acid in the muscles both prior to and immediately after slaughter (Adzitey & Nurul, 2011). This results in lower ultimate pH values (pH\textsubscript{24}) in PSE poultry breast meat. Previous studies have also reported higher pH\textsubscript{24} values in normal poultry breast meat in comparison to PSE breast meat (Fраqueza, Cardoso, Ferreira, & Barreto, 2006; Petracci, Betti, Bianchi, & Cavani, 2004; Wilkins, Brown, Phillips, & Warriss, 2000). Petracci, Betti, Bianchi, & Cavani (2004) reported pH\textsubscript{24} values of 5.89 for normal and 5.77 for PSE broiler breast meat. In addition, Fраqueza, Cardoso, Ferreira, & Barreto (2006) reported pH\textsubscript{24} values below 5.8 for PSE turkey breast meat. In the present study, average CIE $L^*\textsubscript{24}$ and $b^*\textsubscript{24}$ color values were lower ($P < 0.05$) in normal chicken breast meat when compared to PSE chicken breast meat samples (Table 4.1). In addition, average CIE $a^*\textsubscript{24}$ values were higher ($P < 0.05$) in normal breast meat when compared to PSE breast meat samples (Table 4.1). Our color results are in agreement with previous studies. For example, Petracci, Betti, Binachi, & Cavani (2004) characterized normal broiler breast meat ($50 \leq L^* \leq 56$) and PSE broiler breast meat ($L^* > 56$). Moreover, Sheard, Hughes, & Jaspal (2012) characterized broiler breast fillets as normal ($L^* 53-57$), PSE ($L^* > 58$), and dark ($L^* < 52$). Previous studies have examined the reason for pale color appearance in PSE meat (Swatland, 2004, 2008). Swatland (2008) proposed that the myofibrils are birefringent in nature and their
birefringence is inversely proportional to the pH, and the PSE meat appears paler due to greater light scattering in comparison to normal meat.
Table 4.1  pH, color ($L^*$, $a^*$, and $b^*$), cooking loss, and shear force of normal and PSE chicken breast samples

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH$_{24}$</th>
<th>CIE $L^*_{24}$</th>
<th>CIE $a^*_{24}$</th>
<th>CIE $b^*_{24}$</th>
<th>Cooking loss (%)</th>
<th>Shear force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5.85$^a$</td>
<td>56.0$^a$</td>
<td>1.0$^a$</td>
<td>5.7$^a$</td>
<td>20.0$^a$</td>
<td>20.4$^a$</td>
</tr>
<tr>
<td>PSE</td>
<td>5.60$^b$</td>
<td>61.5$^b$</td>
<td>0.6$^b$</td>
<td>7.2$^b$</td>
<td>21.8$^a$</td>
<td>22.7$^b$</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.5</td>
<td>0.09</td>
<td>0.25</td>
<td>0.56</td>
<td>0.5</td>
</tr>
</tbody>
</table>

$^a-b$ Means with the same letter within a column are not different ($P > 0.05$).

[Commission Internationale d’Eclairage (CIE) $L^*_{24}$ (lightness) = 0 to 100; CIE $a^*_{24}$ (redness) = −60 to 60; CIE $b^*_{24}$ (yellowness) = −60 to 60 were measured at 24 h postmortem]
4.3.2 Cooking loss

No differences \((P > 0.05)\) in cooking loss values were observed between normal and PSE chicken breast meat samples (Table 4.1). This was unexpected, since the lower pH in PSE breast meat is usually associated with higher cooking loss. Woelfel & Sams (2001) reported higher cooking in PSE (26.3%) and lower cooking loss in normal (23.6%) broiler breast fillets collected from a commercial processing plant. In addition, Woelfel, Owens, Hirschler, Martinez-Dawson, & Sams (2002) reported higher cooking loss values in PSE (26.4%) and lower cooking loss in normal (21.0%) broiler breast meat from a commercial processing plant. In the aforementioned studies, normal and PSE breast meat samples were characterized based on \(L^*\) values, pH, and expressible moisture at 24 h postmortem. In the current study, normal and PSE breast fillets were characterized only based on pH and \(L^*\) values measured at 24 h postmortem. In the present study, the lack of difference in cooking loss between normal and PSE breast meat may have been due to the higher purge loss in PSE meat due to packaging and freezing prior to cooking.

4.3.3 Warner Bratzler shear force determination

Shear force values were greater \((P < 0.05)\) for PSE chicken breast samples in comparison to normal chicken breast samples (Table 4.1). Since average shear force values for normal and PSE samples were below 30 N, all of these samples would be considered tender to a large number of consumers (Schilling et al., 2003). Even though all samples were on average tender, 14.8% of the PSE strips that were sheared had a shear force greater than 30 N, indicating that these samples were neither tender nor tough. This was in comparison to 3.0% of strips that were greater than 30 N for normal chicken.
breast. Previous studies have also reported higher shear force values in cooked PSE breast meat when compared to normal breast meat. Zhuang & Savage (2010) reported Warner-Bratzler shear force values of 31.4 ± 15.7 N, 33.3 ± 7.8 N, and 47.0 ± 14.7 N in light (\(L^* > 60\)), medium (55 < \(L^* < 59\)) and dark (\(L^* < 55\)) cooked broiler breast fillets, respectively. Furthermore, Droval et al., (2012) reported shear force values of 24.7 ± 5.7 N in normal and 40.8 ± 11.2 N in PSE cooked broiler breast meat.

**4.3.4 Whole muscle proteome profile**

The image analyses of the whole muscle proteome gels revealed five differentially abundant protein spots in normal and PSE breast (pectoralis major) muscles (Figure 4.1 and Table 4.2). Two protein spots (phosphoglycerate mutase-1 and glycogen phosphorylase) were over-abundant \((P < 0.05)\) in normal breast meat (Table 4.3), whereas the other three protein spots (beta-enolase, fructose-bisphosphate aldolase-C, and myosin heavy chain skeletal muscle) were over-abundant \((P < 0.05)\) in PSE breast meat (Table 4.3). The five differentially abundant proteins identified are involved in glycolysis and muscle contraction (Table 4.3).
Figure 4.1  Comassie stained two-dimensional gel of the whole muscle proteome extracted from Normal and PSE broiler breast (*pectoralis major*) muscles

Five protein spots differentially abundant in normal and PSE breast are encircled and numbered. The spot numbers in the figure correspond to the proteins identified by tandem MS in Table 4.2 and Table 4.3.
Table 4.2  Differentially abundant sarcoplasmic and myofibrillar proteins in normal and PSE chicken breast (*pectoralis major*) muscles identified by Tandem Mass Spectrometry

<table>
<thead>
<tr>
<th>Spot</th>
<th>Accession number</th>
<th>Protein</th>
<th>Species</th>
<th>ProtScore/Matched peptides</th>
<th>Sequence coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Q5ZLN1</td>
<td>Phosphoglycerate mutase 1</td>
<td><em>Gallus gallus</em></td>
<td>12.07/9</td>
<td>46.2</td>
</tr>
<tr>
<td>2</td>
<td>P07322</td>
<td>Beta-enolase</td>
<td><em>Gallus gallus</em></td>
<td>6.17/5</td>
<td>26.3</td>
</tr>
<tr>
<td>3</td>
<td>P11217</td>
<td>Glycogen phosphorylase</td>
<td><em>Homo sapiens</em></td>
<td>10.17/7</td>
<td>17.8</td>
</tr>
<tr>
<td>4</td>
<td>P53449</td>
<td>Fructose-bisphosphate aldolase C</td>
<td><em>Gallus gallus</em></td>
<td>2.28/1</td>
<td>11.7</td>
</tr>
<tr>
<td>5</td>
<td>P13538</td>
<td>Myosin heavy chain skeletal muscle</td>
<td><em>Gallus gallus</em></td>
<td>15.62/7</td>
<td>9.1</td>
</tr>
</tbody>
</table>

For each spot, parameters related to protein identification are provided, including NCBI accession number; species; ProtScore and number of matched peptides; sequence coverage of peptides in tandem mass spectrometry.

*a* Spot numbers refers to the numbered spots in the gel image (Figure 4.1).
Table 4.3  Functional roles of the differentially abundant sarcoplasmic and myofibrillar proteins in normal and PSE chicken breast (*pectoralis major*) muscles identified by Tandem Mass Spectrometry

<table>
<thead>
<tr>
<th>Spot</th>
<th>Protein</th>
<th>Function</th>
<th>Over-abundant treatment</th>
<th>Spot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phosphoglycerate mutase 1</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>1.76&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Beta-enolase</td>
<td>Glycolytic enzyme</td>
<td>PSE</td>
<td>2.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Glycogen phosphorylase</td>
<td>Glycolytic enzyme</td>
<td>Normal</td>
<td>1.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Fructose-bisphosphate aldolase C</td>
<td>Glycolytic enzyme</td>
<td>PSE</td>
<td>1.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Myosin heavy chain skeletal muscle</td>
<td>Muscle contraction</td>
<td>PSE</td>
<td>1.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Spot numbers refers to the numbered spots in gel image (Figure 4.1).

<sup>b</sup> Spot ratio of normal/PSE.

<sup>c</sup> Spot ratio of PSE/normal.
4.3.4.1 Glycolytic enzymes

Two glycolytic enzymes (phosphoglycerate mutase 1 and glycogen phosphorylase) involved in glycolytic metabolism were over-abundant ($P < 0.05$) in the normal group whereas the other two glycolytic enzymes (beta-enolase and fructose-bisphosphate aldolase-C) were over-abundant ($P < 0.05$) in the PSE group (Table 4.3). Poultry muscle fiber types are classified into three main types: Red type (Type 1/Slow-twitch), Intermediate (Type IIa), and White type (Type IIb/Fast-twitch) (Berri, 2000; Foegeding et al., 1996). Poultry breast muscles with a greater proportion of white fibers, have a greater capacity for anaerobic (glycolytic) metabolism, and are fast-fatiguing and therefore used for brief bursts of activity (Foegeding et al., 1996; George & Berger, 1966). In the present study, the majority of the proteins identified in the normal and PSE breast (pectoralis major) muscles were glycolytic enzymes which may be due to their inherent glycolytic muscle fiber composition.

4.3.4.1.1 Phosphoglycerate mutase-1

Phosphoglycerate mutase-1 was over-abundant in normal breast meat (Table 4.3). This enzyme converts 3-phosphoglycreate into 2-phosphoglycerate using 2, 3 biphosphoglycerate as a cofactor in the glycolytic cycle (Qiu et al., 2008). Previous studies have reported the presence of phosphoglycerate mutase-1 in chicken skeletal muscle (Doherty et al., 2004; Mekchay et al., 2010; Teltathum & Mekchay, 2009). Doherty et al., (2004) studied the soluble proteome changes in layer chicken pectoralis muscle from 1 to 27 days after hatching. In this study, increasing the birds’ age from day 1 to 27 resulted in a 44 fold increase in pectoralis muscle mass, which led to a substantial
increase in glycolytic enzymes in the soluble proteome. The major proteins identified in the *pectoralis* muscle soluble proteome were beta-enolase, glyceraldehyde 3-phosphate dehydrogenase, lactate dehydrogenase, triose phosphate isomerase, phosphoglycerate mutase, creatine kinase, pyruvate kinase, and phosphoglycerate kinase. In another investigation, Teltathum & Mekchay (2009) studied the proteome profile changes of Thai indigenous *pectoralis* chicken muscle at 0, 3, 6, and 18 weeks of growth. They reported that five proteins (phosphoglycerate mutase 1, apolipoprotein A1, triose phosphate isomerase 1, heat shock protein, and fatty acid binding protein 3) significantly correlated with chicken age. In particular, phosphoglycerate mutase 1 and triose phosphate isomerase 1 proteins were positively correlated with age of the birds. In addition, Mekchay, Teltathum, Nakasathien, & Pongpaichan (2010) used a proteomic approach to determine the tenderness differences in Thai native and commercial broiler chicken muscles. They reported that pyruvate kinase muscle, phosphoglycerate mutase, and triose phosphate isomerase were 1.3 fold lower in abundance in the low-shear force group of Thai native chicken samples. On the contrary, in the present study, phosphoglycerate mutase-1 was 1.5 fold over-abundant ($P < 0.05$) in normal chicken breast samples than when compared to PSE breast meat (Table 4.3). The differences in the abundance of phosphoglycerate mutase-1 may be due to genetic variability in the chickens, since commercial broiler chickens were utilized in the present study in comparison to Thai native chickens that were evaluated in the referenced study.

### 4.3.4.1.2 Glycogen phosphorylase

Glycogen phosphorylase was over-abundant ($P < 0.05$) in normal breast meat (Table 4.3). Glycogen phosphorylase is rate limiting and one of the first glycolytic
enzymes involved in the glycolytic pathway for conversion of glycogen to glucose (Bollen, Keppens, & Stalmans, 1998; Remignon et al., 2007). Similar to our study, Samuel (2009) investigated the differences in meat quality attributes and protein expression in normal and pale broiler breast meat. Results indicated lower water holding capacity, protein solubility, and pH in the pale broiler breast meat. Moreover, in the same study, the presence of phosphorylase enzyme was reported in the myofibrillar fraction of both normal and PSE broiler breast meat. Pietrzak, Greaser, & Sosnicki (1997) used SDS-PAGE, western blotting, and immunofluorescence microscopy to indicate that phosphorylase became tightly associated with the myofibrils in PSE pectoralis major turkey muscles. In addition, Rathgeber, Pato, Boles, & Shand (1999) detected two glycolytic enzymes (creatine kinase and glycogen phosphorylase) in the myofibrillar protein extract of rapid glycolyzing (pH ≤ 5.8 at 15 min postmortem) turkeys that were not present in normal glycolyzing (pH > 6.0 at 15 min postmortem) turkeys. In contrast to previous studies, in our study, glycogen phosphorylase was over-abundant in normal broiler breast meat (Table 4.3). In the referenced studies, one-dimensional SDS-PAGE was used to determine the protein abundance between normal and PSE turkey breast meat. However, in the current study, proteins were first separated based on their isoelectric points in the first dimension, prior to resolving the proteins in the second dimension on SDS-PAGE gels. Therefore, the over-abundance of glycogen phosphorylase observed in the normal broiler breast meat in the current study could be attributed to the differences in methodology used for determining protein abundance between normal and PSE broiler breast. In addition, differences in the protein abundance of glycogen phosphorylase may have been due to the use of broiler breast meat samples.
instead of the turkey breast meat samples that were evaluated in the referenced studies. Further investigation is needed to elucidate the role of glycogen phosphorylase in accelerated postmortem glycolysis observed in the PSE pectoralis muscles.

4.3.4.1.3 Beta-enolase

In the present study, beta-enolase was over-abundant ($P < 0.05$) in PSE breast meat (Table 4.3). Enolase is a dimeric glycolytic enzyme that converts phosphoglycerate into phosphoenolpyruvate (Keller et al., 2000; Ohlendieck, 2010). Previous research has shown that beta-enolase and glycogen phosphorylase were over-abundant in glycolytic fast-twitch skeletal muscles (Merkulova et al., 2000). Jia et al., (2006) studied the changes in enzymes associated with energy metabolism during early postmortem in longissimus thoracis bovine muscle. The results indicated an over-abundance of the glycolytic enzymes (phosphoglycerate kinase and beta-enolase) in longissimus muscle postmortem. These enzymes contributed to an increased rate of glycolysis and meat quality variation. The over-abundance of beta-enolase enzyme in PSE meat is indicative of increased glycolytic activity and hence a lower pH in PSE breast meat.

4.3.4.1.4 Fructose-bisphosphate aldolase C

Fructose-bisphosphate aldolase C was over-abundant ($P < 0.05$) in the PSE group (Table 4.3). Aldolase with Fructose-1,6-bisphosphate breaks down fructose-1,6-bisphosphate to fructose-6-phosphate (Rakus et al., 2004). Fructose-bisphosphate aldolase C is one of the major enzymes involved in the initial steps of glycolysis (Kijowski, 2001). Previous investigation studied the modification of glycolytic enzymes in normal glycolyzing ($\text{pH}_{20\text{min}} 6.47$) and fast glycolyzing ($\text{pH}_{20\text{min}} 6.04$) turkey breast
muscles and reported differences in two glycolytic enzymes (glyceraldehyde-3-phosphate dehydrogenase and fructose bisphosphate aldolase A) between normal and fast glycolyzing turkey breast muscles (Molette et al., 2005). Furthermore, researchers studied the proteome differences in acid, PSE, and normal pork *longissimus* muscle, and documented that aldolase was one of the most abundant proteins identified from pork *longissimus* muscles (Zelechowska, Przybylski, Jaworska, & Santé-Lhoutellier, 2012). Thus, in the current study, the over-abundance of fructose-bisphosphate aldolase C and beta-enolase are likely related to the accelerated postmortem pH decline and the meat quality differences between normal and PSE chicken breast (*pectoralis major*) muscles.

4.3.4.2 **Myosin heavy chain**

Myosin heavy chain skeletal muscle, one of the major myofibrillar proteins involved in muscle contraction, was over-abundant (*P* < 0.05) in the PSE group (Table 4.3). A myosin molecule consists of two myosin heavy chains, two alkali light chains, and two other light chains (Rathgeber, 2000). Previous research evaluated the influence of myosin light chain and heavy chain isoforms on the early postmortem glycolytic rate and meat quality traits in porcine *longissimus* muscle. In this study, the myosin heavy chain isoform was more abundant in the fast-glycolyzing treatment than the normal-glycolyzing treatment. Moreover, in the same study, the myosin heavy chain (fast/slow) ratio was negatively correlated to the early postmortem muscle pH (*r* = -0.51) (Choi et al., 2007). Similarly, in our study, the over-abundance of myosin heavy chain in the PSE group may be related to postmortem pH. Since myosin has an isoelectric point of 5.4 (Huff-Lonergan & Lonergan, 2005). In low pH meat, myofibrillar proteins are in close proximity to their isoelectric point and lose their ability to hold water (Huff-Lonergan &
In the current study, the average pH of the PSE breast meat samples ranged from 5.4-5.7. The lower pH observed in the PSE breast meat may have resulted in less tender meat after cooking. In the current study, higher Warner-Bratzler shear force values were observed in PSE breast meat samples in comparison to normal breast meat samples (Table 4.1). Moreover, previous studies have suggested the role of myosin isoforms in imparting meat tenderness and quality. For example, Lametsch et al., (2003) examined the postmortem proteome changes in porcine muscles and related the proteome changes to tenderness. In this study, myosin heavy chain fragment spot intensity was negatively correlated ($r = -0.49$ to $-0.54$) with Warner-Bratzler shear force values, whereas myosin light chain II fragment was positively correlated ($r = 0.49$ to 0.59) with Warner-Bratzler shear force values. In a similar study, postmortem proteolysis and its relationship to meat quality was examined in pig longissimus muscle. These researchers reported that myosin light chain 1 and actin were positively correlated ($r = 0.70$ to 0.75) with Warner-Bratzler shear force values (Hwang et al., 2005). Molette, Rémignon, & Babilé (2005) studied the proteomic basis of BUT9 pectoralis major turkey breast muscles and their relationship to meat quality and reported differences in three protein spots between normal and fast glycolyzing turkey breast muscles. These proteins were identified as myosin heavy chain, actin fragments, and glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Thus, in the present study, the over-abundance of myosin heavy chain in the PSE breast meat may be related to shear force differences between normal and PSE breast meat.
4.4 Conclusions

The results of the present study indicate that the over-abundance of glycolytic enzymes and structural proteins in PSE breast meat may be related to the meat quality differences between normal and PSE breast meat. Future studies should consider the proteome in PSE and normal meat drip loss which might further explain the meat quality differences between normal and PSE breast meat. In addition, further studies will focus on correlating differences in the proteome abundance to pH, color \((L^*, a^*, \text{and } b^*)\) and instrumental shear force of normal and PSE broiler breast meat.
CHAPTER V
SUMMARY AND CONCLUSIONS

In the first part of this study, results indicated that over-abundance of proteins involved in glycolytic, muscle contraction, proteolytic, ATP regeneration, energy metabolism, and CO₂ hydration in PSE breast meat may be related to the meat quality differences between normal and PSE breast meat. Based on the protein markers identified in the present study, future studies should focus on identifying the genes responsible for differences in protein abundance and their relationship to poultry meat quality. In addition, further studies will focus on stressing birds of different genetic strains to find out how the protein abundance is related to genetic selection in order to help decrease the incidence of PSE meat and other quality problems in the poultry industry.

In the second part of this study, results indicated that over-abundance of glycolytic enzymes and structural proteins in PSE breast meat may be related to the meat quality differences between normal and PSE breast meat from a commercial processing plant. Future studies should consider the proteome in PSE and normal meat drip loss which might further explain the meat quality differences between normal and PSE breast meat. The differences in the whole (pectoralis major) muscle proteome between the two studies might be due to the broiler samples (controlled research condition vs commercial processing plant) and differences in the bird handling prior to slaughter. Furthermore, woody breast meat, also described as muscle myopathy, is prevalent in the poultry industry.
industry in many countries including the United States. Proteomics could be a successful tool for elucidating the differences in protein abundance due to muscle myopathy.
REFERENCES


