

**Effects of Protein Modification on Textural Properties  
and Water Holding Capacity of Heat Induced  
Turkey Breast Meat Gels**

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## ABSTRACT

The main objectives of this research were to examine effects of protein modification (protein cleavage and crosslinking) on turkey meat gelation and to evaluate textural properties and water holding capacity of meat gels prepared from normal and PSE (pale, soft, exudative) turkey breast meat.

First, the effect of protein degradation on turkey breast meat gelation was studied. To create different extent of proteolysis in the meat,  $\alpha$ -chymotrypsin (EC 3.4.21.1) was added to normal and PSE meat batters at 0, 2.5, 5 and 10 ppm levels. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of cooked meat gels showed progressive protein hydrolysis with increasing enzyme level. Texture profile analysis and torsional analysis of the cooked meat gels showed an incremental deterioration in texture with increasing enzyme level. This inferior texture caused by proteolysis was similar to that observed in the gels made from PSE turkey meat alone. Pearson correlation coefficients indicated gel textural properties and expressible moisture were highly correlated to the degree of proteolysis, especially to that of myosin heavy chain ( $p < 0.001$ ).

The second study focused on modifying protein size to improve meat gelation, especially PSE meat gelation. Transglutaminase (TGase, EC 2.3.2.13) was chosen due to its ability to catalyze crosslinking of proteins. Pea protein isolate, an alternative to soy protein, was also evaluated as a meat protein extender. Textural profile and torsional gelometry analyses of the cooked meat gels showed TGase alone significantly ( $p < 0.05$ ) increased gel texture, especially for those made from PSE meat. However, cook yield of the meat gels was compromised possibly due to steric effects. Addition of

pea protein isolate alone improved cook yield and gel texture, especially for the gels made from PSE meat. The combination of TGase and pea protein produced the strongest meat gels, while maintaining a similar cook yield to the control. SDS-PAGE showed the disappearance of several protein bands contributed from the meat or pea protein with TGase addition, indicating that these likely were crosslinked and too large to enter the gel. Dynamic rheological analysis revealed TGase altered the viscoelastic properties of the meat or meat-pea protein mixtures and produced more elastic gels on cooling.

This research indicated proteolysis had a dramatic impact on textural properties of turkey breast meat gels. Crosslinking of proteins catalyzed by TGase significantly improved gel texture, especially for the gels made from PSE meat. However, TGase-assisted crosslinking of proteins resulted in greater cooking losses unless an extender/adjunct such as pea protein was added.

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## 1. INTRODUCTION

For the past few decades, a significant quality problem has plagued the meat industry. This problem is characterized as meat being pale in color, soft in texture, and with exudative drip loss (PSE). There is a direct monetary loss in the 2-3% drip, which is not merely water but water-soluble nutrients. The most significant detrimental effect to processors is the fact that PSE meat has lower value for further processing due to its poor binding properties (Cassens, 2000).

PSE pork was first reported in the 1950s, and an enormous amount of research work has been devoted to this problem since. It is now known that genetics, diet, pre-slaughter animal handling and the rate of carcass chilling can influence the incidence and magnitude of the PSE condition, and various strategies have been employed to minimize the incidence of PSE pork, such as gene screening, good pre-slaughter handling, quick chilling, and carbon dioxide stunning (Lee & Choi, 1999). However, surveys of the incidence of PSE showed the problem continues to exist today to approximately the same extent as when it was first found (Cassens, 2000; Kauffman *et al.*, 1992). This fact indicates that the development of PSE is a complex process and further investigation from a deeper scientific perspective may be needed to solve this quality problem.

PSE-like poultry meat was identified in the 1960s (Ferket & Foegeding, 1994). With the increase in poultry meat consumption, meat quality defects in poultry have also

risen in recent years. As reviewed by McCurdy *et al.* (1996), the magnitude of the “light meat problem” observed by the turkey industry can range from 5 to 30% depending on the season. As experienced in pig meat, use of turkey meat with PSE properties also has negative consequences for processing in addition to quality issues as fresh meat. Current problems with the soft texture, poor cohesiveness, and poor juiciness of processed turkey breast muscle have resulted in multi-million dollars yearly losses to the turkey industry (Foegeding, 1992).

Recent research has indicated that protein denaturation and degradation early postmortem are major defects of PSE turkey meat (Rathgeber *et al.*, 1999c, 2002). The poor textural properties of processed PSE meat products could be related to the inferior protein functionality in PSE meat. The first part of the present research was to investigate the effects of protein degradation on meat gelation properties. To create various degrees of protein degradation as in PSE meat,  $\alpha$ -chymotrypsin (EC: 3.4.21.1), a serine proteinase, was used to cleave meat proteins.

The aim of the second half of this research was to improve gelation properties of PSE meat. Transglutaminase (TGase; protein-glutamine  $\gamma$ -glutamyl transferase, EC 2.3.2.13) is an enzyme that catalyzes an acyl-transfer reaction between the  $\gamma$ -carboxamide group of peptide-bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptor). TGase has been demonstrated to have the ability to crosslink food proteins of different origins and improve water-holding capacity and textural properties of meat products (Ikura *et al.*, 1992; Kurth & Rogers, 1984; Motoki & Nio, 1983). Milkowski and Sosnicki (1999) filed a patent claiming transglutaminase can improve quality of canned or packaged hams and turkey breasts made from PSE

meat. But no application utilizing transglutaminase in comminuted PSE meat products has been documented. The main objective of the second study was to investigate the effect of TGase on PSE turkey meat gelation.

A variety of non-meat proteins have been used as functional ingredients in comminuted muscle foods to improve the physicochemical characteristics (especially texture-related properties), flavour, and cooking yield (Ramírez-Suárez & Xiong 2003a; Xiong, 2000). Soy proteins are probably the most widely employed non-meat protein additives (Pietrasik & Li-Chan, 2002). However, the application of pea protein, another important member of legume protein family, has not yet been well documented.

The overall objectives of this research were to evaluate textural properties and water holding capacity of meat gels prepared from normal and PSE turkey breast meat, and to investigate the effects of protein modification on turkey meat gelation properties.

## 2. LITERATURE REVIEW

There have been numerous studies on PSE development in pork during the past five decades as reviewed by Cassens (2000). More recently, PSE in poultry has also been studied (Barbut, 1997; Sams & Janky, 1991). It is now known that PSE is not a muscle disease but rather a quality defect. PSE meat exhibits inferior quality characteristics in fresh, cooked, and further processed products. This literature review provides a basic introduction to muscle proteins, the development of PSE, and the mechanism of meat protein gelation. The principles of some texture-related measurements used in this project are also reviewed briefly.

### 2.1 Muscle Proteins

Muscle contains water, protein, lipid, carbohydrate, mineral (ash), vitamins, and nucleic acids (Lawrie, 1991). Muscle proteins not only constitute the major organic compounds of the muscle tissue, but also are responsible for the structural and biological properties of muscle in living animals (Bandman, 1987). Muscle proteins are also affected by the postmortem changes to muscle during its conversion to meat. Moreover, muscle proteins are the principal structural and functional components in processed meat systems (Smyth *et al.*, 1999).

Muscle proteins can be broadly divided into three groups based on their solubility characteristics: sarcoplasmic proteins, the metabolic proteins that are soluble



in water or dilute salt solutions; myofibrillar proteins, the contractile proteins that are soluble in concentrated salt solutions; and stromal proteins, the connective-tissue proteins that are insoluble in both (Lawrie, 1991).

### 2.1.1 Sarcoplasmic Proteins

Sarcoplasmic proteins represent 30-35% of the total muscle proteins or about 5% of the muscle weight (Asghar *et al.*, 1985). Sarcoplasmic proteins can be separated into four different structural components based on sedimentation velocity in differential centrifugation: nuclear, mitochondria, microsomal, and cytoplasmic fractions (Xiong, 1997). There are around 200 different proteins known to be present in the sarcoplasmic fraction, many of which are glycolytic enzymes responsible for the control of enzymatic reactions in muscle (Kijowski, 2001). Despite the various biological functions in muscle, sarcoplasmic proteins exhibit many common physicochemical characteristics. For instance, they have relatively small molecule size, appear globular or rod-shaped in structure, and have low viscosity (Asghar *et al.*, 1985).

Myoglobin is presumably the most important protein of the sarcoplasm because it is responsible for meat colour which is associated with product quality (Kijowski, 2001). As reviewed by Lawrie (1991), the colour of the meat depends not only on the quantity of myoglobin present, but also on the type and chemical state of the myoglobin molecule. Miyaguchi *et al.* (2000) studied the thermal and functional properties of porcine sarcoplasmic proteins, and found that sarcoplasmic proteins had poor water holding properties and formed weak and fragile gels.

### 2.1.2 Myofibrillar Proteins

Myofibrillar proteins are generally extracted in intermediate or high ionic strength buffer, so are referred to as salt-soluble proteins. They constitute about 55-60% of the total muscle protein, or 10% of the weight of the skeletal muscle (Asghar *et al.*, 1985). It is well known that myofibrillar proteins are largely responsible for the textural properties of processed meat products (Asghar *et al.*, 1985; Yasui *et al.*, 1980). The adequate extraction of myofibrillar proteins is particularly important for promoting gel formation in meat products (Li-Chan *et al.*, 1987). Of the myofibrillar proteins, myosin and actin are two major proteins responsible for muscle contraction in the living animal, as well as many functional characteristics in processed meat products.

#### 2.1.2.1 Myosin

The thick myofilaments of the sarcomeres are mainly composed of myosin, which comprises 43-45% of the myofibrillar proteins in the muscle of mammals, birds, and fish (Maruyama, 1985; Robson, 1995; Yates & Greaser, 1983). Myosin is a large fibrous molecule (~ 500 kDa), composed of two large subunits called myosin heavy chains (MHC) and four small subunits called myosin light chains (MLC). The two heavy chains form the rod portion and a large part of the myosin head. The two light chains are located in each of the myosin heads (Bechtel, 1986).

In living muscle, myosin exhibits three important biological properties. First, myosin molecules can assemble themselves and build filaments. Second, the myosin

head has the catalytic site for ATPase activity whose action provides the energy for muscle contraction. Third, myosin forms natural complexes with actin, the major constituent of the thin filament. This interaction is critical for the generation of the force that moves the thick and thin filaments past each other (Stryer, 1995).

Myosin can be extracted with salt (e.g., NaCl, KCl) solutions of concentrations higher than 0.15 M. To prevent simultaneous extraction of actin, MgCl<sub>2</sub> and ATP or pyrophosphate can be added into solutions. Myosin molecules tend to aggregate due to oxidation of thiol groups. The addition of ethylenediaminetetraacetic acid (EDTA) and mercaptoethanol can prevent its aggregation (Kijowski, 2001). The myosin molecule, under the effect of sodium dodecylsulfate (SDS), dissociates into subunits of high and low molecular weight that can be separated by electrophoretic techniques (Table 2.1).

Table 2.1 Myosin subunit molecular weights as determined by SDS-PAGE. Modified from Weeds (1980)

	Fast-twitch myosin (Dalton)	Slow-twitch myosin (Dalton)
Heavy chains	200,000	200,000
Light chains (LC)		
LC-1	25,000	27,500
LC-2	18,000	19,000
LC-3	16,000	–

Because myosin is a large complex protein that has a tendency to aggregate, it would facilitate certain types of studies if myosin molecules are broken down to smaller fragments. One method that has been widely used is to cleave myosin with trypsin, papain, or chymotrypsin (Lowey *et al.*, 1969; Weeds & Pope, 1977). Myosin can be

split by trypsin into functional fragments called light meromyosin (LMM) of 150 kDa and heavy meromyosin (HMM) of 350 kDa. After a prolonged incubation with trypsin, HMM can be further digested to S-1 and S-2 fragments with a molecular weight of 115 kDa (Bechtel, 1986), and 60 kDa (Young *et al.*, 1965), respectively (Figure 2.2). The S-1 subfragment contains an ATPase region, a region of binding actin, and two regions of binding light chains (Bechtel, 1986). The S-1 subfragment has the ability to bind actin thin filaments and generates muscle contraction force (Stryer, 1995). It is suggested that S-1 may play a critical role in the functionality of myosin in processed muscle foods due to its excellent binding capacity (Borejdo, 1983; Borejdo & Assulin, 1980).

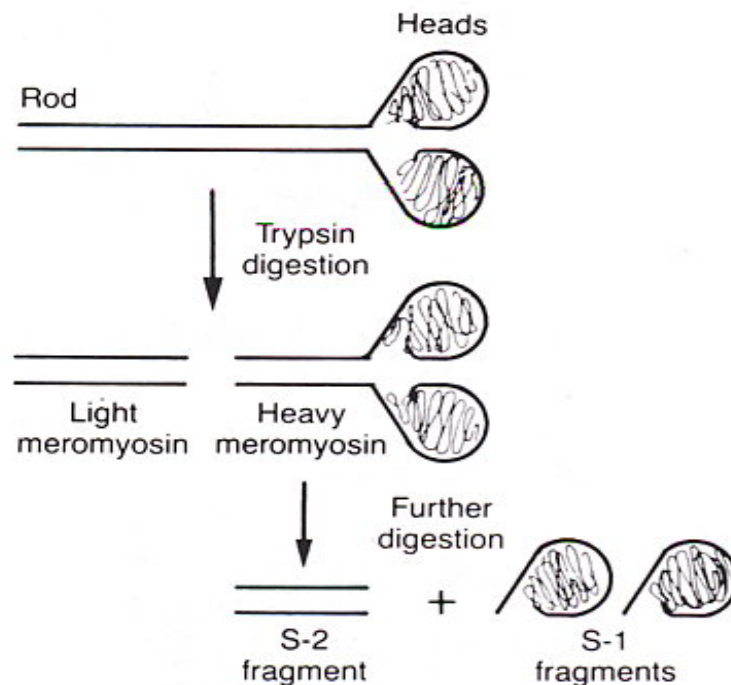


Figure 2.1 Schematic of the generation of LMM, HMM, S-1, and S-2 fragments. Reproduced from Bechtel (1986) with permission.

Myosin contains a large amount of aspartic acid and glutamic acid residues and a fair amount of the basic residues histidine, lysine, and arginine (Harrington, 1979). The isoelectric point of myosin is ~5.3, which means under normal meat processing conditions where the pH value is around 6, the myosin molecule will be negatively charged and have the ability to bind water (Harrington, 1979). Salt will further enhance the water-binding ability of myosin by increasing the effective net negative charge and breaking ionic bonds, causing molecular swelling and water uptake (Acton *et al.*, 1983). The functionality of myosin in processed meat products will be reviewed in the protein gelation section.

#### 2.1.2.2 Actin

Actin is the major constituent of the thin myofilaments and accounts for 22% of the myofibrillar protein (Yates & Greaser, 1983). Actin is a globular protein (G-actin) composed of 376 amino acid residues with a molecular weight of 42 kDa (Kijowski, 2001). Under physiological conditions, G-actin molecules polymerize into a double-stranded fibrous form (F-actin) (Huxley, 1963; Steiner *et al.*, 1952). The F-actin forms the backbone of the thin filament and also provides binding sites for tropomyosin and troponin complex which regulates the activity of myosin ATPase. Actin also has a binding site to myosin. When calcium is present, F-actin comes into contact with the myosin heads of the thick filaments and there is a rapid breakdown of ATP, ultimately resulting in muscle contraction (Bechtel, 1986).

Studies on actin in a model system have revealed that actin alone does not exhibit any binding property (Fukazawa *et al.*, 1961; Samejima *et al.*, 1969). However, in the presence of myosin, actin exerts a “synergistic effect”, thereby considerably complementing the binding characteristics of myosin. This improvement was thought to be due to the formation of the actomyosin complex in the system. Yasui *et al.* (1980) investigated the effects of adding F-actin to myosin preparations and observed the strongest gels were formed when the myosin-actin weight ratio was 2.7.

### 2.1.3 Stromal Proteins

Stromal proteins, also called connective tissue proteins, are salt insoluble in nature. Collagen, elastin, and lipoproteins of the cell membrane, are among the most important connective tissue proteins in the muscle. All of them exhibit a fibrous structure, and in the majority of tissues, collagen quantitatively predominates (Kijowski, 2001). Collagen is generally associated with the toughness of the meat. Collagen is made of three helically twisted polypeptide chains stabilized by intramolecular and intermolecular bonds (e.g., hydrogen bonds). As animals age, more covalent bonds are formed inside and between collagen molecules, which contribute to the toughness of the meat (Asghar *et al.*, 1985; Kijowski, 2001). Ziegler and Acton (1984) indicated that the stromal proteins possessed no gelation ability, as the fraction only coagulated upon heating to 80 °C.

## 2.2 Meat Quality and PSE Meat

Over the past half century, the meat industry has seen a considerable increase in the incidence of pale, soft, exudative (PSE) meat due to the intensive breeding selection for lean muscle development in pigs (Tarrant, 1993). The PSE condition is characterized as being pale in color, soft in texture, and with exudative drip loss. This condition was first found in pig meat, as two-toned color in pork ham muscle had been a controversial issue during the 1950s. As reviewed by Briskey (1964), this phenomenon was originally termed “muscle degeneration” in Denmark, where a research worker Ludvigsen conducted an extensive study in which he described the meat with this condition as having a sour smell, low pH, watery appearance and pale grayish color. Briskey *et al.* (1959) referred to this phenomenon in the United States as pale, soft, and watery tissue. Lawrie (1960) in England described this condition as “white muscle disease”. Numerous research documents about this phenomenon have been reported since then and different terms were given to describe the incidence. Nevertheless, it appears that the present term “pale, soft and exudative” would be a universally accepted term that could be applied to describe this condition in porcine musculature (Briskey, 1964).

Many investigations have been conducted to determine the cause of the PSE problem in pork. As reviewed by Cassens (2000), pigs carrying a so-called porcine stress syndrome (PSS) gene are more susceptible to stress, which may result in higher incidence of PSE than those without this defective gene. PSS gene is a single autosomal recessive gene which, in its homozygous mutant form, can trigger malignant hyperthermia when pigs are under stress (Fujii *et al.*, 1991; MacLennan & Phillips, 1992). The mishandling of the animals prior to slaughter, such as high environmental

temperature, and too short a resting period after transportation, will place stress on the animals and subsequently influence meat quality (Lee & Choi, 1999). These authors also suggested delayed post-slaughter chilling also contributes to the PSE condition.

Conversion of muscle to meat is a complex process during which meat quality is determined. Glycolysis, namely, the breakdown of glucose to produce lactic acid in the absence of oxygen, is the key metabolic pathway early postmortem during the transformation of muscle to meat (Pearson, 1987). Extensive research work has demonstrated that PSE development is the result of accelerated glycolysis triggered by preslaughter stress. This accelerated glycolysis speeds up a series of postmortem changes in PSE muscle. A comparison of the changes in metabolites in normal versus PSE muscle is shown in Table 2.2. Even at the earliest times after death the glycogen level in PSE muscle has been severely depleted and the muscle lactate level is nearly double that in normal muscle. Creatine phosphate (CP) and adenosine triphosphate (ATP) concentrations are also lower at death and both are depleted by 1 h postmortem. The immediate result of this accelerated glycolysis is a rapid pH decline rate. Enfält *et al.* (1993) reported that pig carcasses naturally developing the PSE condition had muscle pH values of below 5.7 at 45 min postmortem, while normal muscle had pH values of above 6.3 at 45 min postmortem.



Table 2.2 Comparison of postmortem levels of metabolites in normal and PSE pig muscle

Metabolite	Normal	PSE
Glycogen, 3 min ( $\mu\text{mol}$ glucose equivalent /g)	35-100	23
180 min ( $\mu\text{mol}$ glucose equivalent /g)	20	0.8
Lactate, 3 min ( $\mu\text{mol}$ /g)	30-40	60
60 min ( $\mu\text{mol}$ /g)	40-60	105
180 min ( $\mu\text{mol}$ /g)	60-80	105
Creatine phosphate, 3 min ( $\mu\text{mol}$ /g)	6.0	3.0
60 min ( $\mu\text{mol}$ /g)	3.0	1.0
180 min ( $\mu\text{mol}$ /g)	2.0	1.0
Adenosine triphosphate, 3 min ( $\mu\text{mol}$ /g)	5.5	3.5
60 min ( $\mu\text{mol}$ /g)	4.5	< 0.5
180 min ( $\mu\text{mol}$ /g)	2.5	< 0.5

Modified from Kastenschmidt *et al.* (1968)

The pH decline is due to the accumulation of lactic acid resulting from anaerobic metabolism, and PSE muscle demonstrates a more rapid pH decline than normal muscle. The rapid metabolism early postmortem results in a low muscle pH while the carcass temperature is still high, leading to the denaturation of muscle proteins. Offer (1991) suggested that denaturation of sarcoplasmic proteins (e.g. myoglobin) in the PSE muscle had a major influence on the increase in paleness, while denaturation of the myofibrillar proteins was responsible for the decrease in water holding capacity. Such protein denaturation leads to the inferior quality characteristics of PSE pork (Bowker *et al.*, 1999). It has been reported that PSE pork muscle has significant lower protein extractability compared to the normal pork muscle (Warner *et al.*, 1997). As reviewed by Bechtel (1986), in extreme cases, muscles with a rapid pH fall while the muscle

temperature is still high may show ~ 50% lower sarcoplasmic protein solubility and 75% lower myofibrillar protein solubility than comparable values for normal meat. In particular, myosin (Penny, 1967) and the sarcoplasmic proteins phosphorylase and creatine kinase (Fischer *et al.*, 1979) are denatured in PSE pork.

Recently, the turkey industry has been faced with an increasing problem similar to PSE conditions found in pork. Barbut (1993 & 1997) and McCurdy *et al.* (1996) observed discoloration in turkey breast meat due to exposure to pre-slaughter stress (struggling or heat), and the pale colour has been correlated with poor water holding capacity and gel strength. Pietrzak *et al.* (1997) reported turkey breast meat that proceeds through rigor at an accelerated rate exhibits reduced protein extractability, water holding capacity, and cook yield. McKee and Sams (1998) suggested elevated post-mortem temperatures contribute to the pale, exudative meat characteristics of turkey breast fillets by accelerating the development of rigor mortis. These studies indicated the denaturation of muscle proteins at low pH and high carcass temperature conditions was responsible for the above-mentioned inferior meat quality. Moreover, Rathgeber *et al.* (1999c) found rapid postmortem glycolysis and delay chilling of turkey carcasses caused myosin degradation. Myofibrillar proteins (e.g. myosin) are important in determining water holding capacity of the myofilament lattice and subsequently play a critical role in protein gelation (Offer & Trinick, 1983). Results of this study (Rathgeber *et al.*, 1999c) provided insight into the molecular basis for previously reported reductions in meat quality of rapid glycolyzing and delay chilled turkey meat. Overall, these studies indicated that turkey breast muscle exhibits various degrees of a PSE condition.

It is generally accepted that the PSE condition is closely associated with pre-slaughter stress. As previously reviewed, meat scientists have identified the porcine stress syndrome (PSS) gene which triggers an abnormal stress response in pigs and subsequently leads to a PSE condition (Lee & Choi, 1999). The syndrome is also triggered following exposure of animals to volatile halogenated anesthetics, such as halothane (Hall *et al.*, 1980). The similar rapid postmortem glycolysis in pigs and turkeys suggests that there may be a defect in either or both of the turkey skeletal muscle ryanodine receptors. Owens *et al.* (2000) conducted a study aimed at identifying a similar mechanism for the triggering of the PSE condition in poultry. At 2-4 weeks of age, turkeys from commercial strains were subjected to 3% halothane gas and classified as either halothane positive (HAL+) or negative (HAL-) based on muscle rigidity within the legs. All birds were raised under the same conditions and then slaughtered at 20 weeks of age. The authors found the incidence of PSE was significantly higher in HAL+ birds compared with HAL-. These results suggested that halothane screening may also be used in predicting the development of PSE meat in poultry. However, Cavitt *et al.* (2004) found halothane sensitivity had no effect on rigor development, muscle colour, or water-holding capacity in the tested broiler strains, which means halothane sensitivity was not able to identify birds prone to developing PSE meat. Nevertheless, other research has found that genetic factors might play an important role in stress susceptibility. Sante *et al.* (1991) showed that the rate of pH decline was faster in a high performance turkey breed compared to a slow growing breed. Dransfield and Sosnicki (1999) explained that higher growth rates may induce morphological abnormalities, induce larger fiber diameters and a higher proportion of glycolytic fibers,

and a lower proteolytic potential in the muscles. After death, the faster development of rigor mortis increases the likelihood of paler colour and reduced water holding capacity and poorer quality of further processed products.

Based on the current appreciation of PSE conditions in poultry, various strategies have been employed to increase the quality of poultry meat, such as good pre-slaughter management, gas stunning (Savenije *et al.*, 2002), and quick chilling (Alvarado & Sams, 2002). However, observations by the turkey industry indicated that the incidence rate of the “light meat problem” (as they refer to it) still could reach as high as 30% (McCurdy *et al.*, 1996). This fact indicates that the development of PSE is a complex process and further study is needed. On the other hand, the existing high PSE occurrence rate requires new processing techniques to improve the poor binding ability of PSE meat.

### 2.3 Thermally Induced Meat Protein Gelation

Gelation, according to Glicksman (1969 & 1982), is “the association or cross-linking of randomly dispersed polymer chains in solution to form a three-dimensional continuous network which immobilizes liquid in the interstitial structure which resists flow under pressure”. Protein gelation is important for imparting desirable sensory characteristics and textural properties in foods. The characteristics of each gel are different and dependent upon factors such as protein concentration, degree of denaturation caused by pH, temperature, ionic strength and/or pressure (Totosaus *et al.*, 2002). Protein gelation can be achieved by many means, among which, heat-induction is the most widely used method.

Upon heating, meat proteins can form a three-dimensional gel network which provides both structural and functional properties to meat products (Acton *et al.*, 1983). Thermally induced gelation involves both intramolecular (conformational) and intermolecular changes in proteins. The mechanism of gel formation may differ among proteins due most likely to the type of molecular interactions that stabilize the gel of different protein systems. Functionally, these events involve protein-water interaction, protein-fat interaction and protein-protein interaction (Acton & Dick, 1989). These interactions may consist of multiple hydrogen bonds (Eldridge & Ferry, 1954), and disulfide linkages (Huggins *et al.*, 1951) or peptide bonds (Bello, 1965). They may also involve electrostatic and hydrophobic interactions (Wolf & Tamura, 1969). Ferry (1948) proposed an interpretation for protein gelling: firstly, the unfolding or dissociation of protein molecules is provoked by heat or other means, followed by the second step in which the association and aggregation reactions result in a gel system. It is important that the rate of the second step remains slower than the first one, because protein aggregation will then be ordered enough to allow gel formation (Kinsella *et al.*, 1994).

Of the three major protein groups in muscle, myofibrillar proteins are the most important to the ultimate development of the gel structure in heat-processed products (Smith, 1988). Gelation of myofibrillar proteins is the most important functional property that occurs in restructured, formed and comminuted products and is also responsible for texture, viscoelastic traits, juiciness, and stabilization of fat emulsions in processed meat products (Xiong, 1997).

### 2.3.1 Protein Denaturation

Most proteins, in their native states, are folded into well-defined, usually essentially rigid, three-dimensional structures. For most proteins, this structure is compact and globular, such as myoglobin (Kendrew *et al.*, 1961), lysozyme (Blake *et al.*, 1965), and chymotrypsin (Matthews *et al.*, 1967). In a few proteins the native structure is rod-like, or it may consist of a mixture of rod-like and globular proteins, such as myosin (Woods *et al.*, 1963). The native structure of the protein may undergo conformational changes in response to various conditions. For instance, during food processing, meat proteins may denature due to the exposure to heat, change of the pH and ionic strength, etc.

Kauzmann (1959) proposed denaturation as “a process (or sequence of processes) in which the spatial arrangement of the polypeptide chains within the molecule is changed from that typical of the native protein to a more disordered arrangement.” Tanford (1968) supplemented Kauzmann’s definition by requiring that there be no alteration in the protein’s primary structure. Denaturation can therefore be restricted to the “continuous process of native protein structural changes involving the secondary, tertiary, or quaternary structure during which alteration of hydrogen bonding, hydrophobic interactions, ionic linkages and oxidation-reduction or interchange reactions of covalent disulfide bonds occur without alteration of the amino acid sequence” (Anglemier & Montgomery, 1976). For food scientists, denaturation is of great importance because it has a significant influence on protein functionality, such as water holding capacity, protein solubility, emulsification and gelation.

Proteins are peptide chains composed of amino acids which possess hydrophilic or hydrophobic characteristics depending on the polarity of the side chain. During the formation of protein molecules, native proteins tend to orient the hydrophobic portions into the interior side of the molecule and the hydrophilic portions into the exterior side of the molecule to reach its most stable configuration. Upon heating, the energy imparted to the protein molecules can break the relatively weak forces that hold the proteins in their folded and helical tertiary and secondary configurations. As a result, the protein molecules unfold and the internally directed hydrophobic regions are exposed to the outside of the molecules. This process is called denaturation. When too many hydrophobic sites are exposed, the interactions become inevitable between the exposed hydrophobic sites, resulting in protein aggregation (gelation) (Nakai, 1983).

The thermal denaturation of a protein is usually accompanied by several conformational transitions in structure (Lesiów & Xiong, 2001). Transition temperatures (designated as  $T_m$ ) for various muscle proteins have been used to identify points where conformational changes in the protein occur upon the absorption of thermal energy. The transition temperatures of the major muscle protein, myosin, have been extensively studied (Dudziak & Foegeding, 1988; Liu & Foegeding, 1996; Smyth *et al.*, 1996). It is now generally accepted that the myosin molecule undergoes two major transitions during heating: the first one is denaturation and aggregation of myosin heads, and the second one is disassociation of myosin rods (Burke *et al.*, 1973; Samejima *et al.*, 1976). The variations among transition temperatures reported in the literature were attributed to different experimental conditions such as muscle type, batter composition, ionic strength, and pH (Lesiów & Xiong, 2001). It is also generally accepted that the second

transition (~ 55 °C) is possibly the most crucial, since gels do not attain appreciable strength until this temperature is reached (Ziegler & Acton, 1984).

### 2.3.2 Protein Aggregation

While heating, the denatured, unfolded protein molecules re-orient themselves, interact at specific points, and finally form an ordered three-dimensional network structure (Foegeding, 1988a). On the basis of observations of the heat-induced gelling properties of myosin and its proteolytic subfragments, Samejima *et al.* (1981) proposed that the heat-induced gelation of myosin consists of two reactions: (1) aggregation of the globular head segments of the myosin molecule, which is closely associated with the oxidation of sulfhydryl groups; and (2) network formation of the unfolded helical tail segments. In addition, the authors suggested that the head portions associate to form “super-junctions” which provide extra cross-linking to the gel network.

Although the mechanism of gel formation may differ among proteins, it appears that the heat-induced gelation occurs in two phases. At temperatures below 55 °C, the major events are changes in protein conformation (denaturation). The subsequent aggregation and gelation begin at approximately 55 °C when the myosin rods start to aggregate. It is essential that the rate of the aggregation remains lower than the denaturation step to allow an ordered gel formation (Totosaus *et al.*, 2002).



### 2.3.3 Factors Affecting Meat Protein Gelation

Protein gelation can be viewed as a process during which protein interact with the surrounding environment. The interactions mainly include protein-water, protein-fat, and protein-protein interactions (Acton & Dick, 1989). These interactions are influenced by factors that can affect protein gelation, as well as affecting the type and properties of gels (Totosaus *et al.*, 2002).

#### 2.3.3.1 Protein Type and Concentration

Many different model systems have been used to study the functional properties of meat proteins. It is now clear that the main proteins responsible for meat product binding are myofibrillar proteins, of which myosin and actin are the most important (Barbut, 2001; Smith, 2001). Myosin and actin have high content of basic, acidic and polar amino acids (Whiting, 1988a). This characteristic contributes to the binding properties and water-holding capacity of comminuted products, forming a desired gel strength system. Different muscle fiber types contribute to different characteristics of protein gelation. For instance, chicken breast muscle myosin formed stronger gels than myosin from leg muscle (Morita *et al.*, 1987), and red muscle myosin formed gel networks composed of finer filamentous structures than white muscle myosin gels (Asghar *et al.*, 1984). It is accepted that discrepancies in gelation between white and red fiber types or muscles are attributed to isoforms of myofibrillar proteins, particularly myosin, and more immediately, to their different protein aggregation patterns during gel formation (Xiong, 1994). Protein concentration is the major factor in determining

fracture properties and water holding capacity in protein gels (Hongsprabhas & Barbut, 1997). There must be a minimal concentration of the protein itself, below which a continuous three-dimensional structure cannot be formed (Ferry, 1948).

### 2.3.3.2 Temperature

Temperature is one of the most important factors in gel formation because it is a driving force to unfold protein domains (Totosaus *et al.*, 2002). Several studies have reported an increase in surface hydrophobicity, followed by a decrease in the second stage of thermal gelation (Wang & Smith, 1994). These results indicated the two stages of gel formation, which corresponded to Ferry's (1948) conclusion: unfolding and aggregation stage. First, the unfolding or dissociation of protein molecules provoked by heat, followed by the second step in which the association and aggregation reactions resulted in a gel system. It is important that the rate of the second step remains lower than the first one, because protein aggregation will then be ordered enough to allow gel formation. Wang and Smith (1994) suggested that low temperatures (slow heating rate) favored the aggregation process, whereas high temperatures (fast heating rate) weakened the intramolecular and cross-linking bonds of myosin gels. For example, Camou *et al.* (1989) evaluated the effects of heating rates and protein concentrations on gels made of extracted salt-soluble proteins. They found at all protein concentrations, the slowest heating rate resulted in the greatest gel strength, and gel strength decreased with increasing heating rate. The authors concluded that a slower heating rate allowed proteins to unfold and re-orient themselves and therefore form a more ordered and higher strength gel network.

### 2.3.3.3 Ionic Strength

Myofibrillar proteins are salt-soluble proteins. The addition of salt enhances electrostatic repulsions between protein molecules and therefore increases the interactions between proteins and water, namely, protein extractability, solubility and water binding capacity (Huxley, 1963). Salt concentration plays an important role in the amount of extracted protein and later textural properties of the product. Barbut and Findlay (1989) indicated that reducing the salt level from 2.5 to 1.5% (w/w) in commercial type poultry meat batters could result in a substantially lower final rigidity value of the final products. Commercially 2-3% (w/w) salt is applied in meat products.

Alkaline phosphates are widely used to improve protein functionality in meat products. They can provide high ionic strength to help extract meat proteins, as well as increase the pH about 0.2 units, which is beneficial (Trout & Schmidt, 1986). Phosphates cause the dissociation of the actomyosin complex, the predominant protein complex in postrigor meat (Wang *et al.*, 2005), into actin and myosin (Torigai & Konno, 1996). When phosphate at optimal concentration was added, actomyosin was dissociated and a strong gel network was formed (Ellinger, 1975). The most commonly used phosphates in meat products are sodium acid pyrophosphate (SAPP), tetra sodium pyrophosphate (TSPP), and sodium tripolyphosphate (STPP) (Whiting, 1988b).

### 2.3.3.4 pH Value

The pH of the meat also affects gel formation. At the isoelectric point (pI), proteins have a net charge of 0 and retain the least amount of water. This results in poor

gels or even prevents gel formation (Smith, 2001). Under normal meat processing conditions where the pH value is around 6, myofibrillar proteins will be negatively charged and have the ability to bind water. In muscle tissue exhibiting PSE characteristics, the low pH while meat is still warm denatures proteins and therefore causes poor gel texture. Xiong and Brekke (1991) indicated that the optimum pH for gelation of chicken muscle in 0.6 M NaCl (or KCl) was about 6.0 for breast myofibrils and 5.5 for leg myofibrils.

#### 2.3.3.5 Non-Meat Protein Additives

Non-meat proteins are extensively used in formulated meat products to improve yield and texture by enhancing water-binding properties (Pietrasik *et al.*, 2007). For instance, whey and caseinate have high viscosity in solution that can contribute to water and fat binding (Ramírez-Suárez & Xiong, 2002). Other protein substitutes such as soy proteins can be used as water binders and possible gelling agents to enhance the emulsion stability upon heating (Renkema & van Vliet, 2002). However, most of the non-meat protein components undergo very little structural changes under normal meat processing conditions (65-73 °C, pH 5.5-6.0, and ionic strength 0.1-0.6). For example,  $\beta$ -lactoglobulin, the most abundant constituent in whey protein, denatures at about 80 °C (Ramírez-Suárez & Xiong, 2002), and glycinin from soy protein denatures at 90-94 °C (Ramírez-Suárez & Xiong, 2003a). Hence, there usually is a lack of interaction between non-meat proteins and muscle proteins in processed meat proteins. As a result, these non-meat proteins do not participate in the protein structure or can even negatively

affect texture by a diluting effect or by interference with the gelation of the myofibrillar proteins (Foegeding & Lanier, 1989).

As an important member in the legume family, pea proteins have not received adequate attention in both research and industrial applications. The protein fraction extracted from field peas is a valuable ingredient with a high nutritional value and useful functional properties such as emulsification and film forming ability. Pea protein is neutral in taste and colour and can be incorporated into a variety of food products (Qi & Hydamaka, 2004). It was one of the objectives in the present study to evaluate the functionality of pea proteins in a comminuted meat system.

#### 2.3.3.6 Endogenous and Exogenous Enzymes

Gel weakening, commonly known as modori, occurs in many fish protein gels during thermal processing. The primary cause for this gel weakening is believed to be certain enzymes that hydrolyze myofibrillar proteins, especially myosin, into small fragments, and consequently affects gel properties (Wasson, 1992). Similar gel-softening phenomena in materials prepared from low-grade meat or meat byproducts were observed from beef and pork (Park *et al.*, 1996; Wang & Xiong, 1998). More recently, Rathgeber *et al.* (1999c & 2002) have found early postmortem myosin degradation in PSE pork and poultry meat and indicated gels with impaired texture and water holding capacity result when made from PSE meat.

There are a number of ingredients that can be used to modify or improve the rheology and textural properties of protein gels. Transglutaminase has been one of the agents widely used in recent studies. Transglutaminase (amine  $\gamma$ -glutamyl transferase,

EC 2.3.2.13) catalyzes a protein cross-linking reaction through an acyl transferase mechanism involving protein-bound glutamyl residues (acyl donor) and primary amines (acyl acceptors), including the  $\epsilon$ -amino group of lysine residues in certain proteins (De Jong & Koppelman, 2002). The covalent cross-linking of proteins catalyzed by transglutaminase can cause dramatic changes in the size, conformation, stability, and other properties of proteins (Motoki & Nio, 1983). The enzyme has been used for modifying the functionalities of various proteins including soy proteins, myosin, gluten, globulin, casein, whey, and pea proteins (Abourmahmoud & Savello, 1990; Siu *et al.*, 2002; Takinami *et al.*, 1984; Truong *et al.*, 2004; Ya, 2004). Addition of 0.1-0.3% (w/w) of transglutaminase may be applied to improve textural characteristics and product cohesiveness (Keeton, 2001). Due to its excellent cross-linking ability, transglutaminase can be used as a binding agent in products where salt reduction is desired (Chin & Chung, 2003; Tsao *et al.*, 2002). Milkowski and Sosnicki (1999) demonstrated that transglutaminase can improve quality of canned or packaged hams and turkey breasts made from PSE meat. But no application utilizing transglutaminase in comminuted PSE meat products has been documented.

#### 2.3.3.7 Processing Factors

The time and temperature of chopping and mixing of meat batter must be carefully monitored during processing. Excessive chopping can lead to protein denaturation due to the heat generated from chopping. In practice, low temperature chopping and mixing are used to help extraction of salt-soluble proteins and avoid melting of fat droplets (Keeton, 2001).

It must be emphasized that gelation is a complex process that involves the incorporation of all the factors mentioned above. The optimum formulation and processing techniques are product-dependent.

#### 2.4 Methods Used to Measure Gelation Texture and Rheology

It is indisputable that texture is an important attribute affecting consumer acceptance of food (Szczesniak, 1998). Texture, by definition, “is the sensory and functional manifestation of the structural and mechanical properties of foods, detected through the senses of vision, hearing, touch, and kinesthetics” (Szczesniak, 1963 & 1998). Thus, it is only the human being that can perceive, describe and quantify texture. Furthermore, it is generally recognized that texture, just like flavour, is a multi-parameter attribute (Szczesniak, 1987). Szczesniak (1963) also suggested textural characteristics of the food could be grouped into three main classes: mechanical, geometrical, and other characteristics. According to Szczesniak (1963), mechanical characteristics are the most important in determining the organoleptic properties of food in the mouth.

The traditional sensory evaluation of food quality involves substantial time and money expenditures, and often exhibits poor reproducibility. There has been a demand both in designing instrumental tests that could predict consumer acceptance, and in having instrumentation that could replace descriptive panels (Szczesniak, 1987). The history of efforts to measure texture by instrumental methods can be dated back to 1861

(Bourne, 1982). Instrumental methods for texture measurement can be divided into three classes, i.e., empirical, imitative, and fundamental tests (Bourne, 1978).

Empirical tests include a series of tests that have been found from practical experience to be correlated with textural quality (Bourne, 1978). A number of instruments have been developed that give good correlations with sensory evaluation of texture on a limited number of foods, but these instruments are not considered precise due to the arbitrary test conditions and the results are difficult to compare with those obtained from more rigorous procedures (Rao & Skinner, 1995).

Imitative tests are tests that attempt to imitate the conditions to which the food is chewed in the mouth or cut on the plate (Bourne, 1978). It is in this area that textural profile analysis (TPA) falls. TPA is a useful tool for obtaining a general indication of texture and for making comparisons. A number of instruments such as the General Foods Texturometer (Friedman *et al.*, 1963), the Instron Universal Testing Machine (Bourne, 1978), and the Texture Analyzer (Szczesniak, 1998), have been developed and are widely used to measure a variety of food products. A detailed discussion is provided in section 2.4.1.

Fundamental tests measure properties that reflect mechanical characteristics of the testing sample such as ultimate strength, shear stress, and various moduli (Bourne, 1978). Large strain methods, such as torsional gelometry and small strain methods, such as dynamic oscillatory rheology, which will be discussed in section 2.4.2 and 2.4.3, are examples of fundamental tests.



#### 2.4.1 Texture Profile Analysis (TPA)

The origin of TPA was derived from Szczesniak's classification of textural characteristics (Szczesniak, 1963). The key principle was to serve as a bridge between the instrumental and sensory evaluation of texture. The major breakthrough came with the development of the General Foods (GF) Texturometer (Friedman *et al.*, 1963). This equipment was designed to simulate the masticating action of the human mouth. A typical TPA test uses a small flat-bottomed cylinder to compress a bite-size piece of food, usually a cube approximately 1.2 cm along each side, to 25% of its original height (75% compression) two times in a reciprocating motion that imitates the action of the jaw. By means of strain gauges and a strip-chart recorder, a force-time curve that portrays the entire force history of the simulated masticatory action is plotted. Nowadays, modern computer-assisted texturometers can directly obtain all TPA parameters by means of its software, providing much faster and more precise results than those with manual readout (Pons, 1996).

Bourne (1978) modified the definitions of some of the TPA parameters proposed by Szczesniak (1963). The definitions of the parameters (shown in Figure 2.3) are listed as follows:

- a. Fracturability (originally called brittleness), defined as “the force at the first significant break in the curve”.
- b. Hardness, defined as “the peak force during the first compression cycle”.
- c. Cohesiveness, defined as “the ratio of the positive force area during the second compression portion to that during the first compression (area 2 / area 1), excluding the areas under the decompression portion in each cycle”.

d. Adhesiveness, defined as “the negative force area for the first bite, representing the work necessary to pull the plunger away from the food sample”.

e. Springiness (originally called elasticity), defined as “the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite”.

f. Gumminess, defined as “the product of hardness x cohesiveness”.

g. Chewiness, defined as “the product of gumminess x springiness”.

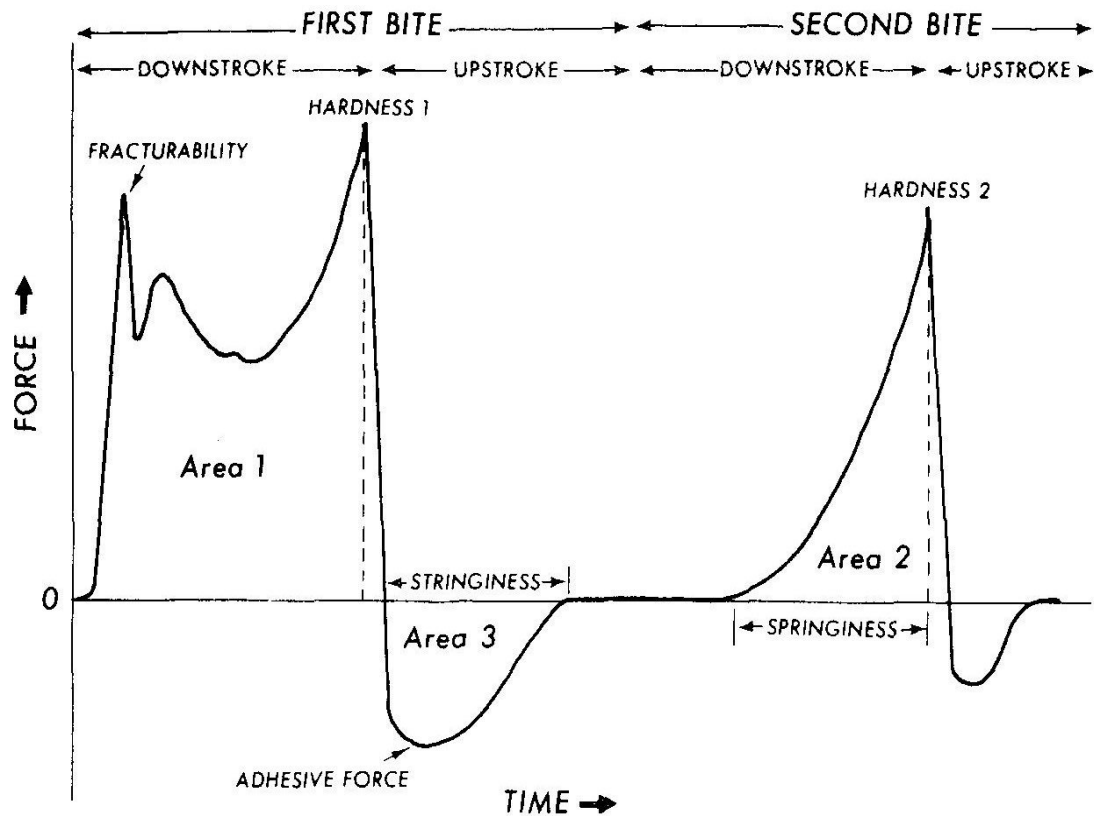


Figure 2.2 Generalized TPA curve obtained from the Instron Universal Testing Machine. Reproduced from Bourne *et al.* (1978) with permission.

Bourne's procedure has been adopted by many popular instruments for objective texture analysis and the basis for practically all the subsequent instrumental TPA studies (Pons, 1996). Instrumental TPA has been extensively used to evaluate the texture of various protein gels, such as sausages (Ziegler *et al.*, 1987), soy protein gels (Lin *et al.*, 2000), low-fat bologna (Shand, 2000), and pork batters with transglutaminase treatment (Pietrasik & Li-Chan, 2002). However, compression is not always suitable for highly deformable elastic materials because failure cannot be achieved. Moreover, the compressive forces applied may dictate the failure mode of the specimen and cause specimen slumping due to water excretion (Truong & Daubert, 2001). When performing TPA, attention should be paid to proper selection of testing and sampling conditions in order to obtain reliable data (Pons, 1996).

#### 2.4.2 Torsional Gelometry

Torsion analysis is widely used for textural measurements of fruits, vegetables, seafoods, and other foods including protein gels (Diehl *et al.*, 1979; Foegeding *et al.*, 1998; Lanier, 1986). In torsional gelometry, a capstan-shaped specimen is twisted, and the generated stress and strain are measured up to the point of material fracture, describing structural changes and textural properties of the tested material (Hamann, 1991). Shear stress is the force applied in parallel to the sample surface, and the corresponding deformation of the test sample is called shear strain (Daubert & Foegeding, 1998). Shear stress is strongly influenced by protein types and concentrations, processing conditions, and ingredients. Shear strain is affected mainly by protein quality and may be considered an evaluation of protein functionality

(Hamann, 1988). Since shear stress and shear strain can be measured independently of one another, a texture map can be plotted using shear stress against shear strain (Figure 2.4). Information from a texture map is useful since it provides “insight into ingredient effects and impact of processing conditions on mechanical properties relating to product texture” (Truong & Daubert, 2001). This torsion texture map could be used for practical applications in the food industry for quality control and new product development.

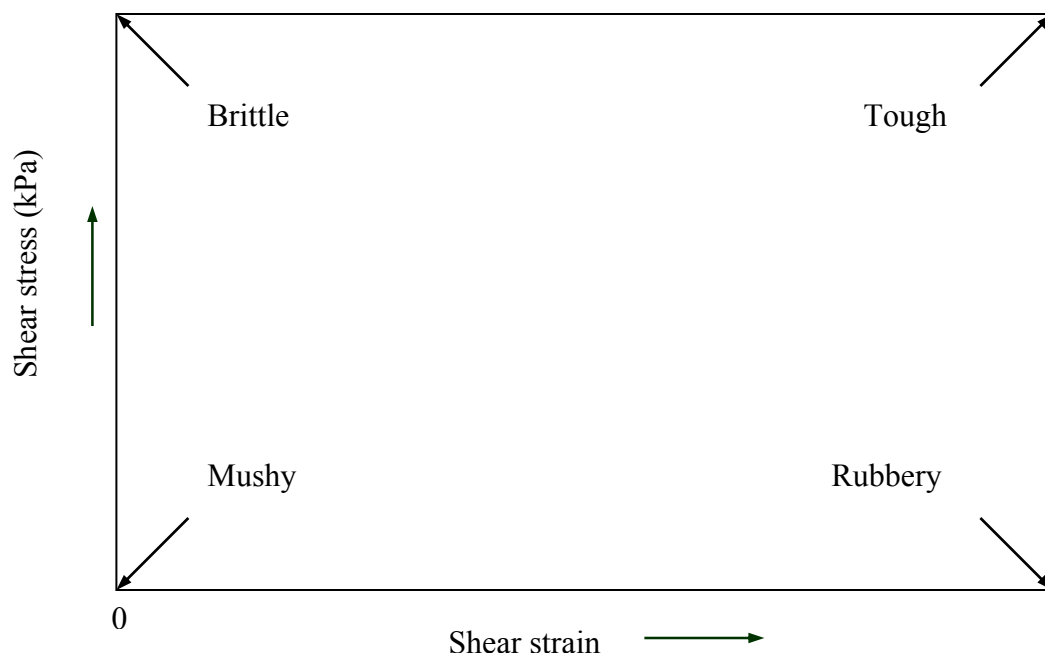


Figure 2.3 A typical torsional texture map. Modified from Lanier (1986).

For gels, TPA hardness and shear stress at failure are highly correlated, whereas shear strain correlated with TPA cohesiveness (Shie & Park, 1999). According to Claus (1995), using a single TPA method for assessing textural characteristics is not sufficient; other instrumental measurements, such as the torsional gelometry test, should be used to conform observations found with TPA or sensory evaluation.

### 2.4.3 Dynamic Oscillatory Rheology

Rheology is the study of the “deformation and flow of material under well-defined conditions” (Hamann, 1988). Rheological properties of gels can be determined at low strains (deformations), where care is taken to prevent sample destruction. These conditions permit a dynamic measure of viscoelastic behaviour which can be monitored with respect to time and temperature (Hamann, 1987). In dynamic rheology, low frequency oscillations are used so as to avoid structural damage and the parameters reflect molecular structures rather than a response to destruction (Savoie & Arntfield, 1996). Rheological analysis can determine rigidity or shear modulus (stress/strain), storage modulus (describes the elastic nature of the material), and loss modulus (describes the viscous nature of the material), which are physical properties of the material that are not dependent on sample size or shape (Foegeding, 1988b). From the perspective of globular protein gelation, the increase in storage modulus has been attributed to increased cross-linking within the network. Increases in loss modulus reflect the increased protein-protein interactions without formation of an elastic structure (Arntfield *et al.*, 1990).

While the evaluation of rheological properties at non-destructive strains is beneficial in understanding gelation, the results are not always pertinent to textural properties perceived by sensory analysis (Hamann, 1987). This is because sensory texture is determined by deforming the sample to fracture.

### 3. MATERIALS AND METHODS

#### 3.1 Chemicals

The  $\alpha$ -chymotrypsin (EC 3.4.21.1) was purchased from Sigma Chemical Co. (St. Louis, MO). Transglutaminase (EC 2.3.2.13, Aactiva® TI, 99% maltodextrin and 1% TGase, contains 100 units of enzyme activity per gram powder) was a gift from Ajinomoto (Ajinomoto USA Inc., Teaneck, NJ) and distributed by Thomas Large & Singer Inc. (Woodbridge, Ontario, Canada). Pea protein isolate (PPI) was provided by Nutri-Pea Limited (Portage la Prairie, Manitoba, Canada). All other chemical reagents were of analytical grade.

#### 3.2 Meat Sample Selection

Tom turkey carcasses weighing about 9-10 kg were selected from a commercial processing line at Lilydale Inc., Edmonton, Alberta. A portable pH meter (Hanna Instruments, Sigma Chemical Co., St. Louis, MO) equipped with a spear tip electrode was used to measure muscle pH. The electrode was inserted into the *Pectoralis major* muscle at the edge of the feather tract directly ventral to the wing joint. Carcasses with a 20 min postmortem pH < 5.8 and > 6.0 were initially classified as PSE and normal, respectively. A sample core of 5 g was taken (using a 22 mm diameter coring device) at 20 min postmortem next to the location of pH measurement and frozen in liquid

nitrogen for later use. The carcasses were immersed in ice water for 2 h (until the temperature of carcasses was below 10 °C), packed with ice, and then transferred to the University of Saskatchewan. The temperature of the carcasses was maintained at ~5 °C during transportation.

Upon arrival at the university (~12 h postmortem), breast meat (*Pectoralis major*) was immediately removed from carcasses and then cut into half to obtain left and right sides. After a drip loss test and measurement of the meat surface colour (described below), the meat was trimmed of connective tissue and fat, vacuum packed in polyethylene storage bags (400 x 300 x 0.15 mm) using a Bizerba Model RD 66 vacuum packing machine (Bizerba Canada, Mississauga, Ontario) set at the maximum vacuum level (seal time 2 sec), and stored in a – 30 °C freezer until use.

### 3.3 Classification of Normal and PSE Turkey Meat

#### 3.3.1 Muscle pH Measurement

##### 3.3.1.1 Iodoacetate pH

Iodoacetate pH of meat samples was measured in duplicate according to Jeacocke (1977). Approximately 2 g of each frozen core sample (frozen at 20 min postmortem) was homogenized with a buffer consisting of 5 mM sodium iodoacetate (brought to pH 7 with 0.1 M potassium hydroxide) at a ratio of 1:10 to arrest glycolysis. Homogenization was carried out with a polytron (PT-MR 3100, Kinematica Inc., Switzerland) on #6 setting (15,000 rpm) in a 50 ml Falcon polypropylene conical tube for 20 seconds. The pH of the homogenate was then measured with a pH meter equipped

with an Accumet temperature-compensated pH electrode (Fisher Scientific, Nepean, Ontario).

#### 3.3.1.2 Ultimate pH

Muscle ultimate pH was measured according to Solomon (1987) with modification. At 48 h postmortem, 20 g of breast meat sample was taken for slurry pH determination. The meat was blended (high speed on blend setting) with 80 ml deionized water for 60 seconds (Osterizer 12 speed blender, Sunbeam Corporation Ltd., Canada). The pH of the mixture was measured using an pH meter equipped with an Accumet temperature-compensated pH electrode (Fisher Scientific, Nepean, Ontario).

#### 3.3.2 Assessment of Drip Loss

At 12 h postmortem, left and right sides of breast meat (*Pectoralis major*) from each bird were separately set on a soaker pad (Ultra Zap®, Paperpak Holdings Ltd., USA) on individual Styrofoam® trays, wrapped with plastic film, and stored at 4 °C for 24 h. The meat was weighed before and after 24 h storage and drip loss was calculated as the percentage of weight loss.

#### 3.3.3 Colour Measurement

Immediately after the drip loss test, which was at ~36 h postmortem, the colour of the meat was measured in duplicate on the intact *Pectoralis major* muscle on the left side of each bird at the point where the *Pectoralis minor* was attached. The instrument



(HunterLab MiniScan, Reston, VA, USA) was set to measure CIE L\*, a\*, b\* using illuminant A and 10° standard observer. Before use, the instrument was standardized using white and black tiles. A duplicate measurement was taken at a 90° angle to the first measurement.

#### 3.3.4 Classification of Normal and PSE Meat

Measurements of pH and drip loss were used to confirm the classification of normal and PSE turkey meat based on the following categories:

For study 1:

Normal: probe pH > 6.0, iodoacetate pH > 6.2, drip loss < 0.7%

PSE: probe pH < 5.8, iodoacetate pH < 6.1, drip loss > 0.7%

Seven birds matching the criteria were selected from each group.

For study 2:

Normal: probe pH > 6.0, iodoacetate pH > 6.0, drip loss < 0.5%

PSE: probe pH < 5.7, iodoacetate pH < 5.8, drip loss > 0.7%

Four birds matching the criteria were selected from each group.

#### 3.4 Proximate Chemical Composition of Raw Meat

Ground breast meat from each bird was analyzed for proximate chemical composition according to AOAC methods (AOAC, 1990). Namely, moisture was determined by measuring weight loss after drying in a 105 °C oven for 16-18 h (AOAC 950.46, 1990), protein content was determined using the Kjeldahl method (AOAC

981.10, 1990), and crude fat was determined using petroleum ether as the solvent (AOAC 960.39, 1990). Protein content of pea protein isolate (PPI) was also measured by the Kjeldahl method. The nitrogen-to-protein conversion factor was 6.25. All samples were analyzed in duplicate.

### 3.5 Enzymatic Assay of Chymotrypsin

The activity of chymotrypsin was measured according to the procedure provided with the purchased product (Sigma Chemical Co., St. Louis, MO). N-benzoyl-L-tyrosine ethyl ester (BTEE) can be hydrolyzed by chymotrypsin and the hydrolysate N-benzoyl-L-tyrosine can absorb ultraviolet light at 256 nm. The absorbance was continuously monitored and the rate of change was used to calculate enzyme activity. One unit of chymotrypsin can hydrolyze 1.0  $\mu$ mole of BTEE per minute at pH 7.8 at 25 °C. The activity of chymotrypsin was measured once a month throughout the study.

### 3.6 Preparation of Meat Gels

#### 3.6.1 Meat Batter Preparation

The vacuum-packed breast meat from each bird was first thawed at  $-1$  °C overnight and then ground through a plate with 3 mm diameter orifices before use. Temperature after grinding remained below 2 °C. For study 1, the formulation included turkey breast meat, sodium chloride (NaCl), sodium tripolyphosphate (STPP), and deionized water (DW). The formulations are shown in Table 3.1. This resulted in meat batters with ~ 14% protein.

Table 3.1 Formulations for study 1 (%, w/w).

Formulation	Meat	NaCl	STPP <sup>1</sup>	Chymotrypsin	DW <sup>2</sup>
Control	60	3	0.3	0	36.7
2.5 ppm	60	3	0.3	2.5 ppm	36.7
5 ppm	60	3	0.3	5 ppm	36.7
10 ppm	60	3	0.3	10 ppm	36.7

<sup>1</sup>STPP: sodium tripolyphosphate

<sup>2</sup>DW: deionized water

Ground meat and non-meat ingredients (salt, STPP and water) were placed in the food processor (Braun UK100, Germany) and then mixed for 3 x 80 sec at speed 2. Then the meat batter was transferred into a plastic bag and vacuum pulled (Komet Vacuboy, Germany) at maximum level to remove air bubbles. For enzyme treatments,  $\alpha$ -chymotrypsin was first made into stock solution (10 mg/ml), and then appropriate quantities of the chymotrypsin solution was added into the formulations at an enzyme/batter ratio of 2.5 ppm, 5 ppm and 10 ppm level on a weight basis. The mixing and vacuuming procedures were the same as described above. Each meat batter size was 500 g. The enzyme solution was made fresh each time. Enzyme levels applied were determined during preliminary work to result in different degrees of proteolysis.

For study 2, the ingredients in the meat batter formulations included turkey breast meat, NaCl, STPP, pea protein isolate (PPI), transglutaminase (TGase), and DW. Specific compositions of each meat batter are shown in Table 3.2.

Table 3.2 Formulations for study 2 (% w/w).

Formulation	Meat	NaCl	STPP <sup>1</sup>	PPI <sup>2</sup>	TGase <sup>3</sup>	DW <sup>4</sup>
Treatment A	42	2	0.3	0	0	55.7
Treatment B	33	2	0.3	0	0	64.7
Treatment C	33	2	0.3	0	0.5	64.2
Treatment D	33	2	0.3	2.6	0	62.1
Treatment E	33	2	0.3	2.6	0.5	61.6
Treatment F	33	2	0.3	2.6	0.5	61.6

<sup>1</sup>STPP: sodium tripolyphosphate

<sup>2</sup>PPI: pea protein isolate

<sup>3</sup>TGase: transglutaminase

<sup>4</sup>DW: deionized water

According to proximate analysis, the protein content of turkey breast meat was 24%, and that of PPI was 76% (as is basis). The particular ingredient percentage in each formulation was selected to provide 10% meat protein (42% meat), 8% meat protein (33% meat), and 2% pea protein (2.6% PPI) to the formulation (Table 3.2). TGase level (0.5%) was chosen according to the guidelines recommended by the supplier and confirmed during preliminary experiments. Ground meat and non-meat ingredients (salt, STPP, PPI, TGase, and water) were mixed as previously described in study 1. For treatment F, PPI and TGase were dispersed in ~ 80 g water and incubated at 50 °C for 30 min before mixing with meat and other ingredients. After mixing the meat batter was degassed using the vacuum method as previously described. Each meat batter size was 500 g.

### 3.6.2 Meat Gel Preparation

For study 1, each batter was stuffed manually into 50 ml Falcon polypropylene conical tubes (30 x 115 mm), centrifuged (Beckman, J2-HC, USA) at 910 g (2,000 rpm) at 4 °C for 5 min to further remove air bubbles. After centrifugation, the tubes (generally 6 per batch) were weighed and incubated in a 40 °C water bath for 15 min to bring the temperature of the stuffed meat batter to 37 °C, which is the optimal temperature for chymotrypsin activity (Simpson *et al.*, 1998). The temperature of the water bath was adjusted to 37 °C and the batter matter was incubated for another 15 min to ensure the function of the enzyme, and then cooked in a water bath (set at 80 °C) for 20 min to achieve a final internal temperature of 80 °C. Chymotrypsin was inactivated during heating (Simpson *et al.*, 1998). After cooking, the tubes were set in ice water for 1 h to cool and kept in a 4 °C refrigerator overnight.

For study 2, each batter was handled the same way as in study 1 described above. After centrifugation, the tubes (generally 6 per batch) were weighed and stored in a 4 °C fridge overnight. The next day the raw batters were cooked the same way as in study 1. TGase was inactivated during heating (Kuraishi *et al.*, 2001). After cooking, the tubes were stored as in study 1.

## 3.7 Evaluation of Water Holding Capacity (WHC)

### 3.7.1 Assessment of Cook Loss

For both study 1 and 2, following overnight storage, chilled gels were removed from the Falcon tubes, blotted with paper towels and weighed. Overall cook loss was

calculated as a percentage based on the raw stuffed batter weight. The average cook loss of six tubes was calculated for each replicate (bird).

### 3.7.2 Determination of Expressible Moisture (EM)

EM measurement of cooked gels was modified from Jauregui *et al.* (1981) as reported by Trout (1988). Cooked gel samples ( $1.5 \pm 0.3$  g) were cut into cylinders by using a 12 mm diameter coring device to keep the shape and size consistent. The gel cylinders were then placed in a Falcon 50 ml tube fitted with a thimble consisting of two layers of Whatman # 3 filter papers (90 mm) folded around one piece of Whatman # 50 (70 mm) filter paper. The tubes were capped and then centrifuged at 228 g (1,000 rpm) (Beckman, J2-HC, USA) for 10 min at 4 °C. EM was expressed as a percentage of the moisture released to original sample weight. Each treatment was measured in triplicate for each replicate.

## 3.8 Methods Used to Measure Gel Texture and Rheology

### 3.8.1 Texture Profile Analysis (TPA)

TPA was determined using a Food Technology Corporation TMS-TP Texture Press (Rockville, MD, USA). Cooked gel samples were cut into cylinders (22 mm in diameter and 15 mm in height) and left to equilibrate at room temperature for 60 min. Then gel samples were compressed to 25% of original height (75% compression) for 2 cycles at a speed of 100 mm/min using a 50 lb transducer (Shand, 2000). Data were

recorded and analyzed automatically by software provided with the machine. Six samples of each treatment were tested.

### 3.8.2 Torsional Gelometry Analysis

Torsional gelometry was measured according to the method described by Rathgeber *et al.* (1999a). Cyanoacrylate glue (Loctite® 404, Loctite Corporation, Rocky Hill, CT) was used to attach styrene discs to both ends of the prepared gel cores (15 mm in diameter, 30 mm in height). The capstan-shaped samples were kept at 4 °C for at least 60 min (to facilitate milling) before being milled into dumbbell-shaped samples using a modified bench grinder. The final specimen had a center diameter of 10 mm. Before testing, the samples were placed in plastic bags (to prevent moisture loss) and left to equilibrate at room temperature for 30 min. Samples were twisted to fracture with a torsion gelometer (Brookfield digital viscometer, Model DV-I, Brookfield Engineering Labs Inc., Stoughton, MA, U.S.A.) at a speed of 2.5 rpm. Shear stress and strain values at failure were calculated on the basis of torque and angular displacement (Hamann, 1983).

### 3.8.3 Dynamic Rheological Analysis

Sample preparation for rheological analysis was similar to the procedure described above in meat batter preparation except for the protein level (meat level) was reduced by half in order to reduce the interference caused by air bubbles trapped in the meat batter. The reduced meat portion was replaced with deionized water.

Heat-induced structural changes in meat batters were monitored by using an AR 1000 rheometer (TA Instruments, New Castle, DE). Samples were loaded in the space (1 mm gap, covered with paraffin oil to prevent moisture evaporation) between the parallel plates and heated from 25 ~ 80 °C at a rate of 1 °C/min and then cooled down to 20 °C at a rate of 5 °C/min. The samples were continuously monitored in an oscillatory mode at a fixed frequency of 1 Hz with a maximum strain of 0.02 (Ramírez-Suárez & Xiong 2003a). Changes in the storage modulus ( $G'$ , rigidity due to elastic response of the testing material) were recorded throughout the heating and cooling process.

### 3.9 Protein Extractability of Meat Gels

#### 3.9.1 Protein Extraction

Protein extraction was performed according to De Backer-Royer *et al.* (1992) with modifications. The cooked meat gel was ground with a mortar and pestle. Approximately 0.5 g of each gel sample was mixed with 5 ml extraction buffer (8 M urea and 0.6 M sodium chloride, ratio 1:10) and extracted for 12 h at 4 °C. Samples were continually stirred with a magnetic stirring bar. The extraction solution was centrifuged (Eppendorf 5415C) at 16,000 g for 10 min and the supernatant was kept at -20 °C for electrophoresis.

#### 3.9.2 Protein Assay

The protein content of the extract supernatant was determined according to the Bradford procedure (Bradford, 1976) using a kit provided by Bio-Rad (Bio-Rad



Laboratories, Inc., Hercules, CA). Bovine serum albumin (BSA) was used as the standard. Measurements were done in duplicate.

### 3.10 Analysis of Protein Profiles of Cooked Meat Gels

#### 3.10.1 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed to investigate protein degradation (study 1) and protein crosslinking (study 2). The procedures were modified from Rathgeber *et al.* (1999b) and Pérez-Mateos *et al.* (2002). A Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA) mini slab gel unit was used as the electrophoresis device. Acrylamide concentration of the resolving gel and stacking gel was 12.5% and 5% (study 1), and 10% and 5% (study 2), respectively. Samples were loaded at a level of 10 µg of protein per lane. Electrophoresis was run at 176 v constant voltage, until the dye front reached the bottom of the frame. Gels were stained for 15 min in 0.1% coomassie brilliant blue R-250, 10% acetic acid, and 50% methanol. Gels were then destained in 10% acetic acid and 7.5% methanol solution until the background was clear. A standard molecular weight marker (Bio-Rad Laboratories, Inc., Hercules, CA) with molecular weight ranging from 6.5 ~ 205 kDa was used.

#### 3.10.2 Gel Image Analysis

Protein profiles of electrophoresis gels were scanned using a densitometer (Model GS 700, Bio-Rad Laboratories, Inc.) and quantitatively analyzed with Quantity

One® software (Version 4.6, Bio-Rad Laboratories, Inc., Hercules, CA). Peak density was chosen as an indicator of the protein content of each band.

### 3.11 Statistical Analysis

Both study 1 and 2 were a split plot in a completely random design. Analysis of variance included quality group (normal and PSE for both studies) and treatment (4 chymotrypsin levels for study 1, and 6 treatments for study 2). Analysis of variance was conducted using the General Linear Models procedure of Statistical Analysis System (SAS 9.1, SAS Institute Inc.) for Windows. Data were presented as mean values. In addition to the analysis of variance of the overall experiment, data sets were split by quality group or treatment level and rerun as one way ANOVA. Duncan's procedure was used to carry out multiple comparisons. There were seven replicates in each quality group (normal and PSE) for study 1, and four replicates in each quality group for study 2.

## 4. RESULTS AND DISCUSSION

### 4.1 Study 1. Effect of Proteolysis on Textural Properties and Water Holding Capacity of Heat Induced Turkey Breast Meat Gels

#### 4.1.1 Classification of Normal and PSE Meat

After animal death, the metabolism shifts from aerobic to anaerobic respiration in muscle tissues due to the unavailability of oxygen carried by blood circulation. The transformation of muscle to meat is a complex process during which meat quality is affected. Due to the rapid glycolysis rate, PSE muscle exhibits different characteristic changes in pH decline rate, colour, and drip loss, which can be used to identify PSE from normal meat (Warner *et al.*, 1997).

Fourteen turkeys were eventually chosen (twenty-one were initially selected based on probe pH) and classified into two categories according to the following criteria:

Normal: probe pH > 6.0, iodoacetate pH > 6.2, drip loss < 0.7% and

PSE: probe pH < 5.8, iodoacetate pH < 6.1, drip loss > 0.7%

Measurements of pH are fundamental to the study of meat quality. As shown in Table 4.1.1, probe pH and iodoacetate pH of the PSE group were significantly lower ( $p < 0.05$ ) than that of the normal group at 20 min postmortem, which indicated the PSE meat had undergone an accelerated glycolysis compared to normal meat. Owens *et al.* (2000) reported pH dropped to 5.72 at 1.5 h postmortem in PSE turkey breast muscle

while normal turkey breast muscle still maintained pH at 6.09. The iodoacetate pH tended to be higher than probe pH. Bendall and Wismer-Pedersen (1962) attributed this discrepancy to the difference in sample temperature at the time of measurement (i.e. probe pH in a warm muscle on the carcass at 37-40 °C vs. iodoacetate pH at 20 °C in this study). They suggested that the effect was mainly due to changes in the  $K_a$  values (dissociation constant) of charged groups on the muscle proteins. When temperature rises, the  $K_a$  value increases, and so does the hydrogen ion content in the solution, therefore pH drops. At 48 h postmortem, there was no significant difference ( $p > 0.05$ ) in ultimate pH between normal and PSE groups, which means there was a similar amount of glycogen in the muscle that had been converted to lactic acid.

Table 4.1.1 Quality measurements of normal and PSE turkey breast meat used in study 1

Measurements	Normal (n = 7)	PSE (n = 7)	LSD <sup>1</sup>	P
Probe pH (20 min)	6.20 ± 0.17 a	5.72 ± 0.12 b	0.17	0.001
Iodoacetate pH (20 min)	6.30 ± 0.11 a	5.96 ± 0.14 b	0.15	0.001
Ultimate pH (48 h)	5.82 ± 0.08 a	5.72 ± 0.10 a	0.14	0.072
Drip loss %	0.48 ± 0.13 b	0.85 ± 0.11 a	0.10	0.001
Colour L* (lightness)	57.46 ± 1.97 a	57.73 ± 1.74 a	2.16	0.793
a* (redness)	12.85 ± 0.81 a	13.08 ± 0.89 a	0.99	0.620
b* (yellowness)	11.43 ± 0.65 a	11.88 ± 0.70 a	0.78	0.234

<sup>1</sup>LSD: least significant difference

<sup>ab</sup> Within the same row, means with different letters are significantly different ( $p < 0.05$ ).

PSE turkey meat showed significantly higher drip loss ( $p < 0.05$ ) than normal meat (Table 4.1.1), which was consistent with that reported by McKee and Sams (1998)

and Wynveen *et al.* (1999) for turkeys, indicating poor water-holding capacity of PSE muscle. Myosin denaturation has been suggested to be the cause of the high rate of drip loss in PSE pork (Offer, 1991). Denaturation of myosin results in shrinkage of the myosin head, drawing the thick and thin filaments closely together. This shrinkage, in addition to the shrinkage of the myofilaments due to the low ultimate pH in PSE pork, can result in more fluid being expelled between fibers and fiber bundles (Irving *et al.*, 1989).

Pale colour is an obvious characteristic of PSE pork. Lawrie (1991) stated in PSE meat, the meat colour pigment – myoglobin is exposed to low pH conditions and becomes oxidized to metmyoglobin which has a low colour intensity. In addition, the surface structure of the meat becomes more “open” under the influence of lower pH and scatters light. McCurdy *et al.* (1996) and Wynveen *et al.* (1999) found that some turkey breast meat had significantly higher lightness ( $P < 0.05$ ) than others. Barbut (1993) reported lightness in turkey breast meat could be correlated with cook loss ( $r = 0.87$ ) and gel strength ( $r = -0.84$ ) of the final products. However, Rathgeber *et al.* (1999a) did not observe any colour difference in ground turkey breast meat between normal and designated PSE muscle. In the present study (Table 4.1.1), no difference in colour was observed ( $p > 0.05$ ) in turkey breast meat between normal and PSE group, possibly because the sample size was not great enough to show the subtle difference (14 samples in the current study vs. thousands of samples in studies conducted by McCurdy *et al.* (1996) and Wynveen *et al.* (1999)).

#### 4.1.2 Proximate Analysis of Raw Meat

As shown in Table 4.1.2, the raw meat from normal and PSE turkeys was similar ( $P > 0.05$ ) in protein, moisture and fat content. These values were within the typical range for turkey according to the USDA National Nutrient Database for Standard Reference, Release 20 (2007). The pH values of raw meat from these two groups were essentially the same (shown in Table 4.1.1). These results were desirable, as this assured that the starting materials (i.e. the meat) were of similar composition and allowed comparisons between these two categories later.

Table 4.1.2 Proximate analysis of raw meat from normal and PSE turkey (as is basis)

Measurements	Normal (n=7)		PSE (n=7)		P
	Mean	SE	Mean	SE	
Moisture %	75.33	0.15	75.34	0.33	0.976
Protein %	23.03	0.18	23.02	0.25	0.975
Fat %	0.64	0.12	0.81	0.19	0.490

SE: standard error

#### 4.1.3 Activity of Chymotrypsin

The assays of chymotrypsin were conducted monthly and the results indicated the enzyme activity was about 57-62 units/mg solid, which was close to the claimed 66 units/mg solid on the label. Chymotrypsin solution was made fresh for each replicate to ensure consistent enzymatic activity.

The optimal conditions for maximum activity of chymotrypsin are 37 °C at pH 8.0 according to the information provided by the supplier (Sigma Chemical Co., St.

Louis, MO). In this study, it took about 15 ~ 16 min to bring the temperature of the stuffed meat batter from ~ 10 °C up to 37 °C in a 40 °C water bath. Then the temperature of the water bath was adjusted to 37 °C and the batter matter was incubated for another 15 min to ensure the function of the enzyme.

#### 4.1.4 Effect of Meat Quality and Proteolysis on Water Holding Capacity

Data analysis showed there were no interactions ( $p > 0.05$ ) between the two main effects: quality group (normal and PSE) and treatment (chymotrypsin level), for all the measurements (WHC and textural properties) conducted in this study. This result demonstrated the meat (meat proteins) responded to chymotrypsin digestion to the same extent no matter what kind of meat was used (normal or PSE). This leads to the speculation that the conformation of the proteins in both normal and PSE turkey meat was similar that the chymotrypsin had equal accessibility to the proteins in both normal and PSE turkey meat used in this study.

In the present study, cook loss was not significantly different between meat gels made from normal and PSE groups, and within the same group, cook loss did not show significant differences ( $p > 0.05$ ) with different concentrations of chymotrypsin (Table 4.1.3). Woelfel *et al.* (2002) reported PSE broiler breast fillets had significant higher cook loss ( $p < 0.05$ ) than normal ones. But Rathgeber *et al.* (1999a) did not observe any difference in cook yield between rapid and normal glycolyzing turkey meat. This contradiction can be attributed to the different sample preparations and cooking methods used. The former authors used a convection oven to directly cook the intact whole

fillets, where the water expelled from the whole muscle was calculated as cook loss. Whereas in the latter case and the present study, the breast meat was ground and formulated into meat batters, and then cooked in capped tubes. It is possible that the expelled water was re-absorbed by the cooked gels during the storage period before testing. It was also noted that the surface of the cooked gels made from untreated (0 ppm) normal meat was firm and smooth, whereas that from PSE and enzyme-treated meat appeared soft and porous, suggesting inferior textural properties.

Table 4.1.3 Water holding capacity of cooked meat gels made from normal and PSE turkey meat with  $\alpha$ -chymotrypsin treatment

WHC	Chymotrypsin ppm	Normal (n=7)		PSE (n=7)		P value
		Mean	SE	Mean	SE	
Cook loss %	0	3.52 a	0.51	4.26 a	0.68	0.401
	2.5	4.05 a	0.63	4.36 a	0.72	0.751
	5	3.69 a	0.54	4.43 a	0.73	0.431
	10	3.54 a	0.32	4.02 a	0.52	0.448
	P value	0.852		0.816		
Expressible moisture %	0	10.26 b	0.31	11.41 b	0.38	0.038
	2.5	10.73 b	0.32	11.47 b	0.48	0.225
	5	11.00 b	0.22	12.05 b	0.68	0.163
	10	14.53 a	0.69	16.60 a	1.73	0.289
	P value	< .0001		0.0002		

SE: standard error

<sup>ab</sup>Data with different letters in the same column are significantly different ( $p < 0.05$ )

Expressible moisture of meat gels prepared from PSE meat was significantly higher ( $p < 0.05$ ) than that from normal meat for the control treatment (without enzyme



addition), which indicated that gels made from normal meat had greater ability to hold water than PSE meat. Camou and Sebranek (1991) reported similar findings in PSE pork gels, but Rathgeber *et al.* (1999a) found no difference between normal and PSE turkey breast meat gels in terms of expressible moisture. This difference, however, became insignificant upon enzyme addition at 2.5 to 10 ppm (Table 4.1.3). For both normal and PSE meat gels, there was a general trend that expressible moisture increased with enzyme addition, which indicated water was less tightly retained in enzyme-treated protein gels. At 10 ppm level, the values were significantly higher ( $p < 0.05$ ) than the other three levels for both groups. According to Schmidt *et al.* (1981), a meat gel is a “three-dimensional interlinked protein network”. This protein network both physically (due to capillarity) and chemically (such as hydrogen bonds and disulfide bonds) stabilizes water (Schmidt *et al.*, 1981). In this experiment, it was observed that the gels made from chymotrypsin-treated meat tend to have more visible pores on the surface of the gels compared to the untreated samples. It is possible that chymotrypsin cleaved meat proteins (e.g. myosin and actin) to smaller peptides and hence fewer crosslinkings were formed within the protein network. This then resulted in a more open (larger pore size) and less tightly restrained matrix to retain water. Even though there was no difference in cook loss, under certain external force, the less tightly restrained water was expelled out of the protein matrix, as was observed for expressible moisture.

#### 4.1.5 Effect of Meat Quality and Proteolysis on Texture Profile Analysis

The poor texture of PSE meat and its products is well known. Camou and Sebranek (1991) reported gels made from PSE pork showed considerably less

functionality because gel strength was weaker (55% lower) than gels made from normal pork. They suggested that proteins in PSE muscle were less functional, which led to reduced protein extractability and hence poor binding ability. Alvarado and Sams (2004) reported slower chilling rate postmortem contributed to higher incidence of PSE conditions in turkey and subsequently resulted in reduced gel strength.

The results of texture analysis in this study showed that with no enzyme addition, meat gels made from normal turkey breast meat had significantly higher hardness values ( $p < 0.05$ ) than those made from PSE meat (Table 4.1.4). No significant difference ( $p > 0.05$ ) in hardness was observed for 2.5, 5, and 10 ppm enzyme addition between these two groups. It is interesting to note, however, that at 2.5 and 5 ppm levels of chymotrypsin addition, although hardness was not significantly different between normal and PSE meat gels, the relatively low P values ( $p = 0.097$  and  $0.087$ ) indicated a trend that the PSE gels were softer than comparable normal ones; and eventually at 10 ppm level, normal meat gel hardness deteriorated to the similar extent as the PSE meat gels (Table 4.1.4). Within the same group, increasing chymotrypsin addition produced softer gels as the hardness value decreased. For instance, compared to the control (0 ppm), hardness of meat gels decreased 9%, 22%, and 71% under increasing enzyme addition (2.5, 5, and 10 ppm) for the normal group, and that of the PSE group decreased 9%, 22%, and 69%, respectively.

Table 4.1.4 TPA hardness and cohesiveness of cooked meat gels from normal and PSE turkey meat with  $\alpha$ -chymotrypsin treatment

TPA	Chymotrypsin ppm	Normal (n=7)		PSE (n=7)		P value
		Mean	SE	Mean	SE	
Hardness N	0	169.71 a	12.23	132.41 a	9.69	0.034
	2.5	153.91 ab	12.92	120.01 ab	13.68	0.097
	5	131.86 b	12.50	103.35 b	8.82	0.087
	10	49.63 c	4.95	41.07 c	5.75	0.282
	P value	< .0001		< .0001		
Cohesiveness	0	0.34 a	0.02	0.26 a	0.02	0.023
	2.5	0.31 ab	0.03	0.23 ab	0.03	0.074
	5	0.26 b	0.03	0.18 b	0.02	0.074
	10	0.07 c	0.05	0.02 c	0.01	0.319
	P value	< .0001		< .0001		

SE: standard error

<sup>abc</sup>Data with different letters in columns are significantly different ( $p < 0.05$ )

As mentioned in the literature review, myofibrillar proteins such as myosin can be digested by proteolytic enzymes such as trypsin and chymotrypsin (Segal *et al.*, 1967; Weeds & Pope, 1977). Since myosin is the most abundant and most functional muscle protein, any alterations in myosin molecules would be expected to affect texture and water holding capacity of the meat products. Lanier *et al.* (1981) noted a decrease in gel strength in minced fish gels and concluded that a heat-stable proteinase present in the raw material (fish tissue) was responsible for the degradation of myosin, which resulted in textural weakness of processed fish gels. Makinodan and Hujita (1990) showed a similar finding by adding an edible Judas' ear mushroom (*Auricularia auriculajudae* (Fr.) Quel), which has been proven to contain a proteinase, into fish meat

paste during the processing of kamaboko. They concluded that the proteinase that exudes from the mushroom hydrolyzes the fish meat proteins, causing textural degradation of kamaboko. Wang and Xiong (1998, 1999) studied the effect of proteolysis on gelation of a myofibrillar protein concentrate from bovine cardiac muscle. They concluded that prolonged incubation at 50 °C caused marked loss in gel strength and water holding capacity of beef heart surimi and this gel weakening most likely resulted from the action of endogenous proteases which presumably consisted mainly of lysosomal proteases (cathepsins).

Significantly higher ( $p < 0.05$ ) cohesiveness values were also observed for meat gels made from normal turkey breast meat than from those made from PSE meat with no enzyme addition (0 ppm) (Table 4.1.4). There was no significant difference ( $p > 0.05$ ) between gels made from normal and PSE meat at any chymotrypsin addition level, but there was a trend ( $p = 0.074$ ) that the PSE gels were slightly less cohesive than comparable normal ones (at 2.5 and 5 ppm). Within the same quality group, increased chymotrypsin addition produced progressively less cohesive gels.

Cohesiveness, according to Szczesniak (1963), is defined as the “strength of the internal bonds making up the body of the product”. As mentioned in the literature review, it is the protein bonding that is responsible for the textural properties of the processed meat products (Xiong, 1997). According to Samejima *et al.* (1969), any damage to myofibrillar proteins, especially to myosin, will affect textural properties of the product. In this study, at 0 ppm level (control), the lower cohesiveness value (compared to that of normal meat gels) observed in PSE meat gels indicated inferior meat protein quality of PSE meat. Likewise, within the same quality group, as

chymotrypsin addition increased, cohesiveness value decreased, indicating myofibrillar proteins had been digested further by chymotrypsin.

Gelation studies suggest that gels do not reach appreciable strength until the myosin tail portion has undergone helix-coil transformation and subsequent cross-linking and the complete myosin molecule is necessary for attaining appreciable continuity and strength in the protein matrix (Acton & Dick, 1989). As previously discussed, meat proteins especially myosin can be broken down to smaller peptides by hydrolytic enzymes such as trypsin and chymotrypsin. This was confirmed in this study upon observation of the protein profiles on SDS gels (to be discussed in section 4.1.8). These smaller peptides exhibited less functional behavior, and were likely “suspended” in the protein matrix rather than forming a crosslinked structure, consequently leading to impaired gel texture. With 5 ppm chymotrypsin addition, hardness of gels made from normal meat was similar to that of gels made from PSE meat without enzyme treatment. This result demonstrated the inferior quality of PSE meat and also suggested besides protein denaturation, protein degradation could contribute to the impaired gelation in PSE meat.

#### 4.1.6 Effect of Meat Quality and Proteolysis on Torsional Gelometry

The results of torsional gelometry showed that meat gels made from normal turkey breast meat had significantly higher shear stress and strain value ( $p < 0.05$ ) than those made from PSE meat at both 0 (control) and 2.5 ppm enzyme addition, but there was no significant difference ( $p > 0.05$ ) for 5 and 10 ppm enzyme treatment (Table 4.1.5).

Table 4.1.5 Torsional analysis of cooked meat gels from normal and PSE turkey meat with  $\alpha$ -chymotrypsin treatment

Torsional gelometry	Chymotrypsin ppm	Normal (n=7)		PSE (n=7)		P value
		Mean	SE	Mean	SE	
Shear stress at failure kPa	0	47.06 a	2.19	39.39 a	2.63	0.045
	2.5	42.41 b	2.57	34.17 b	2.24	0.033
	5	38.46 b	2.47	31.64 b	2.31	0.067
	10	16.43 c	1.88	12.86 c	1.78	0.194
	P value	< .0001		< .0001		
Shear strain at failure	0	2.02 a	0.06	1.85 a	0.05	0.049
	2.5	1.97 a	0.05	1.76 ab	0.04	0.006
	5	1.71 b	0.06	1.68 b	0.06	0.058
	10	1.28 c	0.14	1.15 c	0.10	0.439
	P value	< .0001		< .0001		

SE: standard error

<sup>abc</sup>Data with different letters in the same column are significantly different ( $p < 0.05$ )

Compared to the control (0 ppm  $\alpha$ -chymotrypsin), shear stress of meat gels decreased 10%, 18%, and 65% with increasing enzyme concentration (2.5, 5, and 10 ppm) for meat from the normal group, and that of gels made from PSE meat decreased 13%, 20%, and 67%, respectively. Within the same group, shear strain value decreased 2%, 15%, and 37% upon increasing enzyme addition for normal group, while these values were 5%, 9%, and 38% for the PSE group, respectively. Rathgeber *et al.* (1999a) reported lower shear strain values at fracture were found in gels made from PSE-like turkey meat compared to normal ones. The current experiment not only confirmed their findings but also found lower stress values at fracture for PSE gels. This fact suggested the proteins in PSE meat formed a softer and less elastic gel than proteins from normal

meat. It has been suggested that protein denaturation is responsible for the poor gelation in PSE meat products (Offer, 1991; Pietrzak *et al.*, 1997). Rathgeber *et al.* (1999b) further demonstrated that myosin degradation could attribute to the poor gelation in PSE meat. In this study, untreated PSE meat gels had a similar shear stress value compared with normal meat gels treated with 5 ppm chymotrypsin, which suggested possible protein degradation in PSE meat used for this study.

#### 4.1.7 Protein Extractability in Cooked Turkey Breast Meat Gels

Loss of protein functionality is the major defect in PSE meat. For instance, low protein extractability is typically found in PSE pork meat (Boles *et al.*, 1992; van Laack *et al.*, 1993). More recently, this problem has been identified in PSE turkey meat (Pietrzak *et al.*, 1997; Rathgeber *et al.*, 1999a & 1999c). However, there is no data regarding protein extractability from cooked PSE meat gels. The current study showed cooked gels made from PSE turkey breast meat had a significantly lower protein extractability than those made from normal turkey breast meat (Table 4.1.6).

Table 4.1.6 Protein extractability in cooked gels made from normal and PSE turkey meat (without  $\alpha$ -chymotrypsin addition)

Measurement	Normal (n = 7)		PSE (n = 7)		P
	Mean	SE	Mean	SE	
Total soluble protein content in extract (mg/ml)	9.43 a	0.43	8.00 b	0.31	0.019

SE: standard error

<sup>ab</sup>Data with different letters are significantly different ( $p < 0.05$ )

According to Irving *et al.* (1989) and Offer *et al.* (1989), shrinkage of myosin heads combined with their denaturation causes the thick and thin filaments to be drawn together more closely at rigor in PSE than in normal meat. Consequently, extracting salts may be less effective in dissociating myosin heads and actin filaments, resulting in a low myosin extractability (Offer, 1991). Pietrzak *et al.* (1997) reported that precipitation of the sarcoplasmic protein phosphorylase onto the myofibrillar proteins also contributed to the reduced solubility of myosin molecule in PSE meat. If such precipitation results in decreased myofibrillar protein solubility, then it could result in decreased water-holding capacity and gel strength (Alvarado & Sams, 2004). The results from the current study for TPA and torsional gelometry provided evidence to support this hypothesis.

The extractability of protein increased ( $p > 0.05$ ) after chymotrypsin digestion (data not shown), which is not surprising because smaller proteins generally have larger accessible surface areas to interact with surrounding solution and result in greater solubility (Ooi *et al.*, 1987).

#### 4.1.8 SDS-PAGE Profile of Cooked Meat Gels

SDS-PAGE was performed to examine the protein profile of the cooked meat gels and densitometry was applied to quantify protein degradation. Peak density is proportional to protein content and therefore used as an indicator of protein degradation. As shown in Figure 4.1.1, overall protein profiles from normal (A) and PSE (B) meat gels are essentially the same. Rathgeber *et al.* (1999b) found myofibrillar protein samples from rapid postmortem glycolyzing (PSE like) turkey breast muscle contained



extra protein bands in SDS-PAGE banding patterns compared to that of normal turkey breast meat samples. Western blot analysis identified these protein fragments were from myosin degradation in PSE meat. However, SDS-PAGE results in the current study did not show myosin degradation in PSE meat. This discrepancy indicated the existence of physiological differences among PSE turkeys and possibly the PSE conditions of the samples used in the current study were less severe than that of the samples used in above-mentioned study (Rathgeber *et al.*, 1999b).

As shown in Figure 4.1.1, some proteins, such as myosin heavy chain (MHC, ~ 200 kDa), actin (~ 42 kDa), 154, 48, 38 and 26 kDa peptide bands were digested by chymotrypsin as the peak density decreased with increasing chymotrypsin concentration (Table 4.1.7). Some proteins (e.g. 94 and 60 kDa bands) were not cleaved by chymotrypsin as there was no density change detected. The content of the 78 kDa protein fragment actually increased, which means it probably was obtained from the breakdown of a larger protein such as MHC upon chymotrypsin digestion.

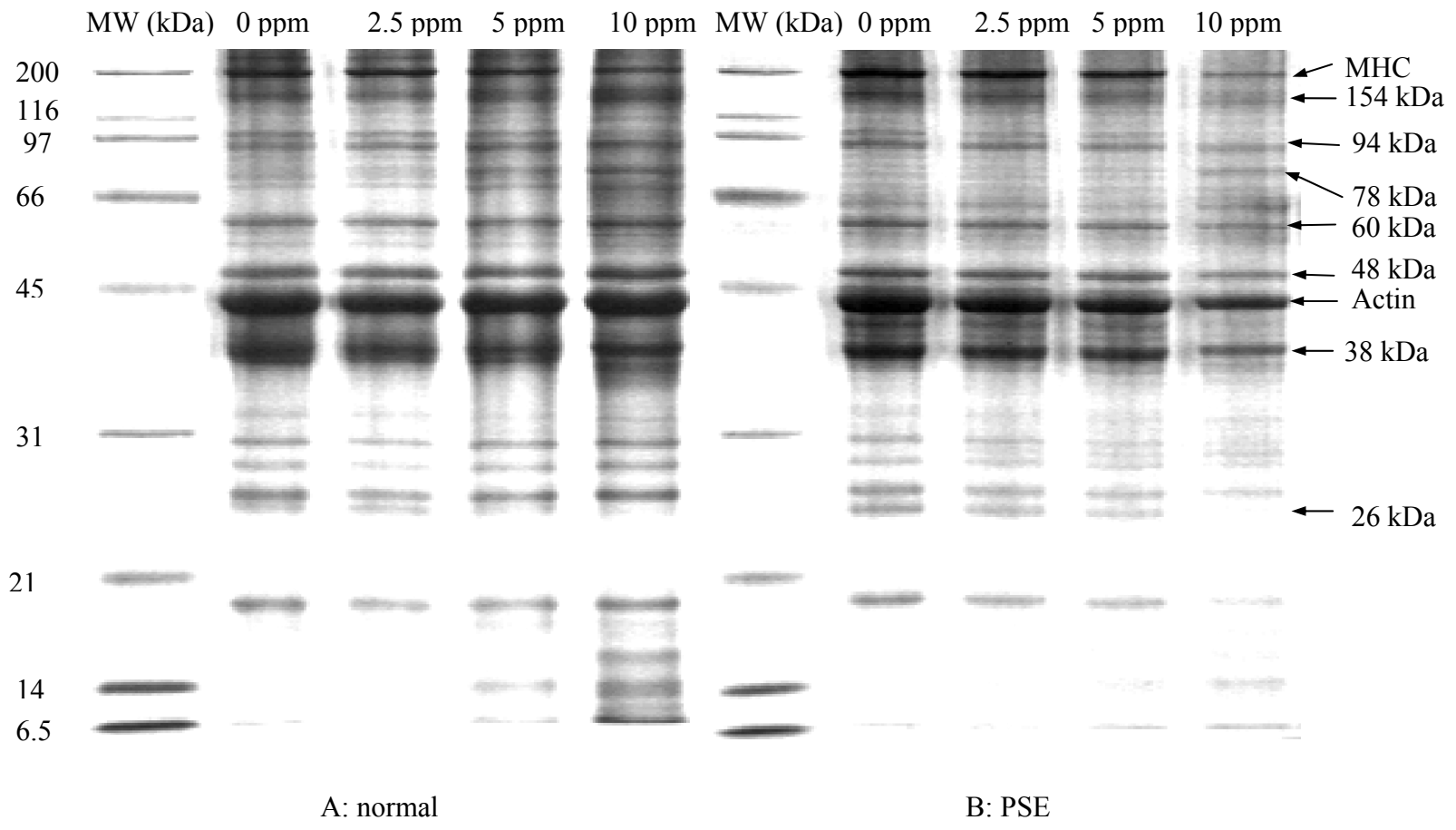


Figure 4.1.1 SDS-PAGE (12.5%) profile of cooked meat gels prepared from normal (A) and PSE (B) turkey breast meat. MW: molecular weight marker. MHC: myosin heavy chain. Each lane contains 10  $\mu$ g of protein. 0-10 ppm: chymotrypsin concentration in the meat batter.

Table 4.1.7 Analysis of peak density\* of major protein bands observed on SDS-PAGE

Measurement	α-chymotrypsin	Normal (n=7)		PSE (n=7)		P value
		Mean	SE	Mean	SE	
~200 kDa (MHC)	0 ppm	0.54 A	0.01	0.53 a	0.03	0.644
	2.5 ppm	0.54 A	0.02	0.54 a	0.01	0.778
	5 ppm	0.49 A	0.02	0.53 a	0.02	0.163
	10 ppm	0.35 B	0.04	0.32 b	0.05	0.661
	P value	< .0001		< .0001		
~154 kDa	0 ppm	0.34 A	0.01	0.34 a	0.03	0.936
	2.5 ppm	0.34 A	0.02	0.33 a	0.02	0.865
	5 ppm	0.32 AB	0.02	0.34 a	0.02	0.447
	10 ppm	0.29 B	0.02	0.24 b	0.04	0.323
	P value	0.032		0.009		
~94 kDa	0 ppm	0.25 A	0.02	0.26 a	0.03	0.915
	2.5 ppm	0.24 A	0.03	0.26 a	0.01	0.532
	5 ppm	0.24 A	0.03	0.27 a	0.02	0.345
	10 ppm	0.26 A	0.03	0.21 a	0.04	0.304
	P value	0.496		0.202		
~78 kDa	0 ppm	0.05 C	0.03	0.08 b	0.03	0.427
	2.5 ppm	0.13 B	0.02	0.10 b	0.03	0.290
	5 ppm	0.15 B	0.02	0.16 a	0.03	0.827
	10 ppm	0.24 A	0.03	0.17 a	0.04	0.189
	P value	< .0001		0.002		
~60 kDa	0 ppm	0.31 A	0.02	0.31 a	0.02	0.833
	2.5 ppm	0.29 A	0.03	0.31 a	0.01	0.618
	5 ppm	0.28 A	0.02	0.30 a	0.02	0.583
	10 ppm	0.30 A	0.03	0.24 b	0.03	0.210
	P value	0.308		0.054		

\* Unit: optical density unit (ODU)

<sup>ABab</sup>Data with different letters in each group are significantly different (p < 0.05)

Table 4.1.7 Analysis of peak density\* of major protein bands observed on SDS-PAGE (continued)

Measurement	$\alpha$ -chymotrypsin	Normal (n=7)		PSE (n=7)		P value
		Mean	SE	Mean	SE	
~48 kDa	0 ppm	0.38 A	0.03	0.38 a	0.02	0.912
	2.5 ppm	0.35 AB	0.03	0.37 a	0.02	0.654
	5 ppm	0.33 B	0.02	0.36 a	0.02	0.396
	10 ppm	0.32 B	0.03	0.27 b	0.03	0.234
	P value	0.011		0.003		
~43 kDa (Actin)	0 ppm	0.55 A	0.02	0.56 a	0.01	0.854
	2.5 ppm	0.55 A	0.02	0.55 a	0.01	1.000
	5 ppm	0.52 B	0.02	0.54 a	0.01	0.344
	10 ppm	0.51 B	0.02	0.47 b	0.03	0.281
	P value	0.001		0.001		
~38 kDa	0 ppm	0.46 A	0.03	0.45 a	0.02	0.730
	2.5 ppm	0.44 AB	0.03	0.44 a	0.01	0.932
	5 ppm	0.42 B	0.03	0.43 a	0.02	0.645
	10 ppm	0.36 C	0.03	0.33 b	0.03	0.564
	P value	< .0001		0.001		
~26 kDa	0 ppm	0.15 A	0.02	0.12 a	0.02	0.208
	2.5 ppm	0.14 A	0.02	0.10 a	0.01	0.148
	5 ppm	0.11 B	0.01	0.10 a	0.01	0.635
	10 ppm	0.04 C	0.02	0.03 b	0.01	0.494
	P value	< .0001		< .0001		

\* Unit: optical density unit (ODU)

<sup>ABab</sup>Data with different letters in each group are significantly different ( $p < 0.05$ )

Results in Table 4.1.7 showed MHC was one of the proteins most affected by chymotrypsin. At 10 ppm chymotrypsin addition level, MHC was hydrolyzed up to 35% and 40% by chymotrypsin for normal and PSE samples, respectively. It is not surprising

that chymotrypsin-treated meat gels exhibited deteriorated texture (as the results of TPA and torsional gelometry analysis have already indicated) since myosin is largely responsible for the textural properties of processed meat products (Asghar *et al.*, 1985; Yasui *et al.*, 1980). The purpose of this part of study was to determine which muscle protein(s) can be hydrolyzed by chymotrypsin and if there is any difference in protein digestibility between normal and PSE meat for chymotrypsin. There is no visible difference in SDS-PAGE protein banding patterns observed for normal and PSE samples. Data in Table 4.1.7 showed there was no significant difference ( $p > 0.05$ ) in band density for individual bands between the gels made from normal and PSE meat at any chymotrypsin level, which suggests chymotrypsin had equal accessibility to the proteins in both normal and PSE meat samples in this study.

#### 4.1.9 Rheology Analysis of Meat Batters Treated with Chymotrypsin

Rheological changes of meat batters treated with chymotrypsin during heating are shown in Figure 4.1.2. The rheograms obtained from normal and PSE samples displayed essentially the same pattern, therefore only one set of data is presented. There was no crossover point observed throughout the whole heating range between storage modulus ( $G'$ ), an indicator of material elasticity, and loss modulus ( $G''$ , data not shown), an indicator of material viscosity. This indicated that there was a network structure formed in the sample prior to rheological testing, and the meat batters exhibited a stronger elastic response than viscous response.

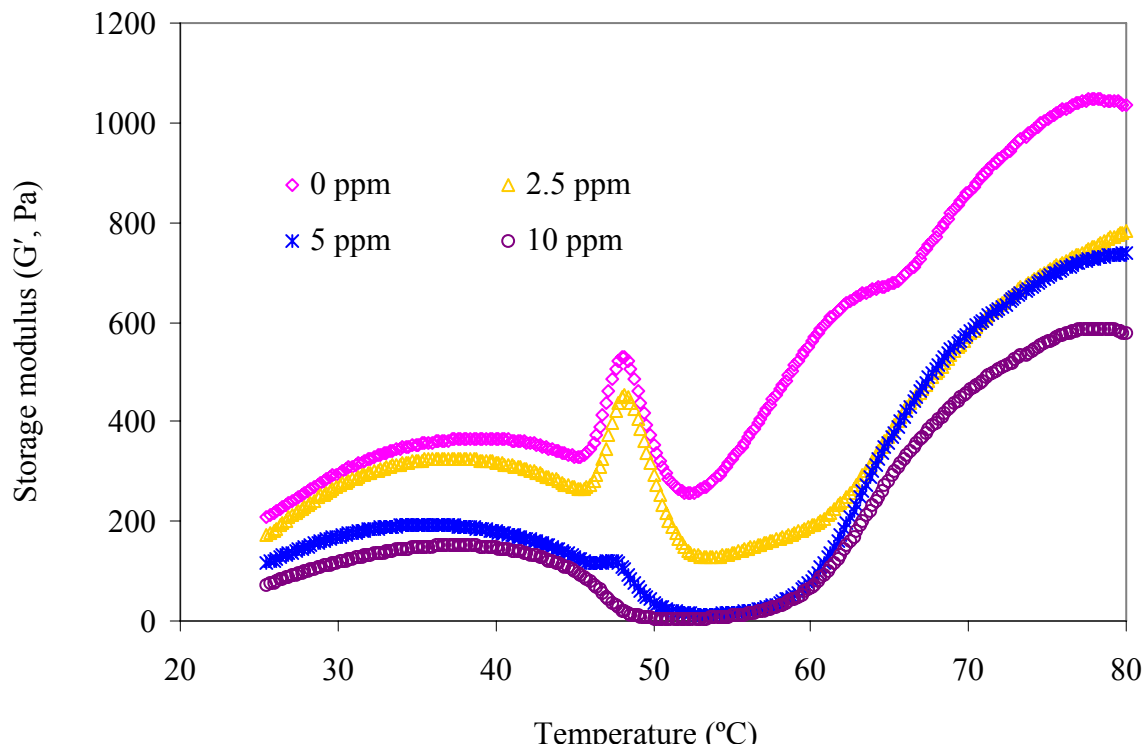


Figure 4.1.2 Rheological changes of meat batters treated with 0 to 10 ppm  $\alpha$ -chymotrypsin during heating. Protein level: 5%. Heating rate: 1 °C/min.

Upon heating, all samples first showed a slow and steady increase in  $G'$ , and then underwent a series of structural changes at the range of 45 ~ 55 °C, after which the  $G'$  continuously increased throughout the rest of the whole heating process.

It was clear that chymotrypsin-treated meat batters had a reduced elasticity even at the beginning of heating (25 °C). The higher the chymotrypsin concentration, the lower the elasticity of the meat batter. For instance, at the beginning of heating (25 °C), with 10 ppm chymotrypsin addition,  $G'$  of meat batter was ~74 Pa, which is almost 1/3 of that (207 Pa) of the control batter (0 ppm). This result indicated meat batter elasticity was affected by the protein's molecular size. As expected, the meat batter composed of

intact myofibrillar proteins exhibited higher elasticity, whereas the chymotrypsin-treated meat batters had progressively lower elasticity as concentration of chymotrypsin increased.

The untreated sample (0 ppm) showed a  $G'$  peak at  $\sim 48$  °C, which is the typical transition caused by denaturation and aggregation of myosin heads during heating (Fernández *et al.*, 1996). It is noted that the treated samples showed either lower peak values (2.5 and 5 ppm chymotrypsin addition) at  $45 \sim 50$  °C or no peak at all (10 ppm chymotrypsin addition), indicating the myosin head region had already been altered by chymotrypsin. The untreated sample also showed a substantially higher  $G'$  value than the treated ones during the second transition ( $\sim 52$  °C) where myosin rods form a more permanent, irreversible complex (Egelandsdal *et al.*, 1986). The treated samples either showed a lower transition  $G'$  value (2.5 ppm chymotrypsin addition) or no clear transition point (5 and 10 ppm chymotrypsin addition). This result suggested that the myosin rods had been cleaved by chymotrypsin and could not form strong crosslinkings during gelation as the intact myosin molecules did. The untreated sample exhibited a much higher  $G'$  value than the treated ones at the end of heating (80 °C), indicating more elastic gels were formed.

#### 4.1.10 Correlation Between Gel Textural Properties and Proteolysis

Table 4.1.8 shows the content of some proteins correlated with meat gel textural properties and water-holding capacity. For instance, intact myosin heavy chain (MHC) and 26 kDa protein content was highly ( $p < 0.001$ ) and positively correlated with TPA

hardness and cohesiveness, shear stress and shear strain, and negatively correlated with expressible moisture. The content of actin (43 kDa), proteins of 154, 48 and 38 kDa size was also positively correlated with gel texture and expressible moisture to a less significant extent. However, the protein fragment with a mass of 79 kDa, which was probably hydrolyzed from higher molecular weight protein(s), had a negative correlation to textural properties and a positive correlation with expressible moisture, indicating breakdown of myofibrillar proteins may be responsible for poor gelation and water holding capacity. This result demonstrated intact meat proteins played a very important role in maintaining textural properties and water-holding capacity of cooked products.

Table 4.1.8 Correlation coefficients between meat gel texture, water holding properties and protein degradation (n = 56)

	TPA Hardness	TPA Cohesiveness	Shear Stress	Shear Strain	Cook Loss	Expressible Moisture
200 kDa (MHC)	0.61***	0.63***	0.67***	0.77***	NS	-0.54***
154 kDa	0.34*	0.36**	0.39**	0.50***	NS	NS
94 kDa	NS	NS	NS	NS	-0.29*	NS
79 kDa	-0.34*	-0.46**	-0.44**	-0.43**	NS	0.38**
60 kDa	NS	NS	NS	NS	NS	NS
48 kDa	0.32*	0.45**	0.47**	0.49**	NS	-0.28*
43 kDa (Actin)	0.44**	0.34*	0.43**	0.44**	NS	NS
38 kDa	0.56***	0.44**	0.57***	0.52***	NS	-0.37**
26 kDa	0.71***	0.55***	0.73***	0.66***	NS	-0.54***

\*, \*\*, and \*\*\* denotes  $p < 0.05$ ,  $0.01$ , and  $0.001$ , respectively. NS means not significant.



#### 4.1.11 Summary of Study 1

PSE problems with the soft texture, poor cohesiveness, and reduced processing yield are major defects facing the turkey industry. While most studies attributed the cause of PSE to protein denaturation (Barbut, 1993 & 1997; McCurdy *et al.*, 1996; McKee & Sams, 1998; Pietrzak *et al.*, 1997), Rathgeber *et al.* (1999b & 2002) indicated degradation of myofibrillar proteins, especially that of myosin may also be responsible for the inferior quality of PSE meat. In this study, in order to create different extents of protein degradation, multiple levels of chymotrypsin addition were chosen to simulate various severities of PSE incidence.

In the current study, gels made from normal turkey meat exhibited textural properties superior to that made from PSE turkey meat due to the inferior protein functionality (e.g. as indicated by excessive drip loss) in PSE meat. Meat gels made from PSE meat showed reduced protein extractability, possibly due to protein denaturation. Upon chymotrypsin treatment, proteins in both normal and PSE turkey meat exhibited proteolysis to varying degrees. Consequently, gel texture increasingly deteriorated with incremental chymotrypsin addition for both quality groups. The changes in rheograms indicated the alternation of myosin heads transition at  $\sim 48$  °C when chymotrypsin was present and the resulting meat gels were less elastic than untreated samples. SDS-PAGE protein profile also revealed muscle proteins such as myosin and actin were digested by chymotrypsin. This study showed the retention of primary structure of muscle proteins, e.g. intact myosin heavy chain, was highly correlated with meat gel textural properties and water-holding capacity.

## **4.2 Study 2. Effect of Protein Crosslinking on Textural Properties and Water Holding Capacity of Heat Induced Turkey Breast Meat Gels**

### 4.2.1 Classification of Normal and PSE Turkey Meat

Twenty-four turkeys were initially selected at the processor based on probe pH values, eight of which were eventually chosen and classified into two categories according to the final criteria:

Normal: probe pH > 6.0, iodoacetate pH > 6.0, drip loss < 0.5% and

PSE: probe pH < 5.7, iodoacetate pH < 5.8, drip loss > 0.7%

These criteria are slightly different from those used in study 1. For instance, the pH cut-off point is lower than in the previous study for the PSE category, and the drip loss cut-off point is lower than in the previous study for selection of birds in the normal category. These criteria indicated a greater difference in raw meat quality between the two quality groups than in the first study, which is beneficial for the purposes of comparing textural characteristics of the products made from these two categories.

As shown in Table 4.2.1, the probe pH and iodoacetate pH of the PSE group were significantly lower ( $p < 0.05$ ) than that of the normal group at 20 min postmortem. There was no significant difference ( $p > 0.05$ ) in ultimate pH between normal and PSE groups. PSE meat showed a significantly higher drip loss ( $p < 0.05$ ) than normal meat. No difference ( $p > 0.05$ ) in raw meat colour ( $L^*$ ,  $a^*$ ,  $b^*$ ) was observed between normal and PSE groups. These results are essentially the same as those in the first study.

Table 4.2.1 Quality measurements of normal and PSE turkey breast meat used in study 2

Measurements	Normal (n = 4)	PSE (n = 4)	LSD <sup>1</sup>	P
Probe pH (20 min)	6.12 ± 0.15 a	5.68 ± 0.04 b	0.19	0.001
Iodoacetate pH (20 min)	6.04 ± 0.05 a	5.77 ± 0.10 b	0.14	0.003
Ultimate pH (48 h)	5.81 ± 0.08 a	5.73 ± 0.11 a	0.16	0.315
Drip loss %	0.36 ± 0.05 b	1.03 ± 0.22 a	0.27	0.001
Colour L* (lightness)	55.18 ± 2.76 a	56.53 ± 1.69 a	3.95	0.435
a* (redness)	12.37 ± 0.96 a	14.54 ± 2.43 a	3.20	0.148
b* (yellowness)	11.84 ± 1.05 a	13.27 ± 2.17 a	2.95	0.282

<sup>1</sup>LSD: least significant difference

<sup>ab</sup>Within the same row, means with different letters are significantly different (p < 0.05).

#### 4.2.2 Proximate Chemical Composition of Raw Turkey Meat

The results of proximate chemical composition of raw turkey breast meat are shown in Table 4.2.2. As observed in study 1, the raw meat from normal and PSE turkeys was similar (p > 0.05) in protein, moisture and fat content.

Table 4.2.2 Proximate analysis of raw meat from normal and PSE turkey (as is basis)

Measurements	Normal (n = 4)		PSE (n = 4)		P
	Mean	SE	Mean	SE	
Moisture %	74.89	0.08	74.58	0.25	0.288
Protein %	23.98	0.20	24.09	0.13	0.659
Fat %	0.44	0.08	0.37	0.02	0.378

SE: standard error

#### 4.2.3 Effect of PPI and TGase on Water Holding Capacity

Cook loss was not significantly different ( $p > 0.05$ ) between normal and PSE meat gels, but within the same quality group, cook loss varied among the treatments (Table 4.2.3). There is a general trend for both normal and PSE groups even though not always significant: the treatment containing 8% meat protein and 0.5% TGase (treatment C) exhibited the highest cook loss, followed by the samples with 8% meat protein (treatment B), and then the samples with 10% meat protein (treatment A), the samples with 8% meat protein, 2% pea protein and 0.5% TGase (treatment E and F), and the samples with 8% meat protein and 2% pea protein (treatment D).

The low cook yield of treatment B can be simply attributed to the low total protein content in the formulation (8% versus 10% in others), and the reason treatment C (8% meat protein + 0.5% TGase) had the lowest cook yield is due, on one hand, to low protein content (8%) and, on the other hand, possibly to the crosslinking of proteins catalyzed by TGase that made the meat matrix structure more compact, therefore expelling more water out of the gel network.

As reviewed by Motoki and Seguroy (1998), TGase has been shown to have the ability to crosslink food proteins from different origins. TGase catalyzes an acyl transfer reaction between a carboxamide of peptide or protein-bound glutamine and a primary amine, forming  $\epsilon$ -( $\gamma$ -glutamyl)lysine bonds between crosslinked proteins. Reports of the effect of TGase on water holding capacity have been controversial. Some researchers have found that TGase enhances water binding capacity in muscle-based products (Pietrasik & Li-Chan, 2002; Tseng *et al.*, 2000). Dondero *et al.* (2006) observed that cook yield of beef meat gels decreased significantly ( $p < 0.05$ ) as TGase level increased,

and they concluded this was probably due to stronger interactions between protein molecules excluding water. The results of the present study confirmed their findings. It was interesting that TGase alone detrimentally decreased cook yield of the meat gels, while a small portion of pea protein in the meat batter formulation significantly increased cook yield. The combination of pea protein and TGase also improved cook yield, especially for products made from PSE meat ( $p < 0.05$ ).

Table 4.2.3 Water holding capacity of cooked meat gels with added PPI and TGase

WHC	Treatment <sup>1</sup>	Normal (n = 4)		PSE (n = 4)		P value
		Mean	SE	Mean	SE	
Cook loss %	A	9.55 c	0.29	10.67 c	1.05	0.343
	B	12.67 b	0.52	13.51 b	1.23	0.555
	C	15.70 a	0.71	18.13 a	1.54	0.202
	D	7.90 c	1.12	5.54 e	1.91	0.327
	E	8.12 c	1.49	7.54 de	2.34	0.842
	F	8.37 c	2.20	8.58 cd	2.62	0.955
	P value		< .0001		< .0001	
EM %	A	15.59 c	0.66	17.13 c	0.95	0.230
	B	21.84 a	0.73	27.48 a	3.02	0.119
	C	20.62 ab	0.84	20.48 bc	0.84	0.906
	D	19.68 b	1.01	22.03 b	1.18	0.181
	E	19.69 b	1.27	20.55 bc	0.97	0.609
	F	21.43 a	1.23	22.17 b	0.98	0.654
	P value		< .0001		0.0007	

SE: standard error

<sup>abcde</sup>Data with different letters in each column are significantly different ( $p < 0.05$ )

<sup>1</sup>A: 10% meat protein; B: 8% meat protein; C: 8% meat protein + 0.5% TGase; D: 8% meat protein + 2% pea protein; E: 8% meat protein + 2% pea protein + 0.5% TGase; F: 8% meat protein + (2% pea protein + 0.5% TGase).

Treatment A, D, E, and F all had the same total protein content in the formulation (10%), however, they exhibited different cook yields after cooking. The general trend for cook loss was A>F>E>D, but there was no significant difference within the normal group. However, there were appreciable differences ( $p < 0.05$ ) among treatments in the PSE group (Table 4.2.3). Samples containing meat and pea proteins (treatment D) had the lowest cook loss probably due to the more open structure (bigger pore size within the meat matrix) resulting from the inclusion of the pea proteins, and therefore this treatment had physically retained more free water within the matrix system. Another possible reason is that pea protein isolate contains some carbohydrates (~ 10%, w/w) such as starch and fiber, which have the ability to interact with the free water (Almond, 2005). For the same reasons, the sample containing meat protein, pea protein and 0.5% TGase (treatment E and F) had lower cook loss than the sample containing meat only (treatment A). However, crosslinking catalyzed by TGase may have made the gel structure more compact (smaller pore size within the meat matrix), so treatment E and F had a slightly higher cook loss than treatment D. But there was no significant difference between treatment E and F, suggesting pre-incubation of pea protein and TGase (treatment F) did not help gels retain more water.

Expressible moisture (EM) data (Table 4.2.3) showed a rather different trend from the cook loss results. Samples containing 8% meat protein (treatment B) had the highest EM value, whereas samples containing 10% meat protein (treatment A) had the lowest. This result demonstrated protein content still played a major role in EM with more water retained under external force at a higher protein content. It was noted that samples containing meat only (treatment A) had a lower EM value than samples

containing meat and PPI (treatment D), as well as samples containing meat, PPI, and TGase (treatment E and F) even though they all contained the same overall protein content. This suggested pea protein had a weaker ability to interact with water verses meat protein, even following crosslinking catalyzed by TGase. It should also be pointed out, however, that treatment A had lost more water during cooking than the other treatments containing the same total protein content (especially for gels made from PSE meat), and therefore there was less moisture to be lost during EM test.

#### 4.2.4 Effect of PPI and TGase on Texture Profile Analysis

The results of textural analysis showed that treatments A and B, the gels made from normal turkey breast meat, had significantly higher hardness values ( $p < 0.05$ ) than those made from PSE meat (Table 4.2.4), which is not surprising as the poor texture of PSE meat and its products is well known. Camou and Sebranek (1991) suggested the proteins in PSE muscle were in a less functional form so meat gels made from PSE meat had a lower gel strength. Compared to study 1, the hardness of untreated normal and PSE meat gels was much lower in the current study, mainly due to the reduced protein content in the formulation (8-10% vs. 14%). This again provides evidence that total protein content is very important to maintain meat product texture.

Table 4.2.4 Texture profile analysis of cooked meat gels with added PPI and TGase

TPA	Treatment <sup>1</sup>	Normal (n = 4)		PSE (n = 4)		P value
		Mean	SE	Mean	SE	
Hardness N	A	62.49 b	1.54	44.16 bc	5.29	0.016
	B	37.08 d	3.07	22.59 d	2.92	0.014
	C	64.72 b	1.19	53.42 b	5.00	0.070
	D	46.15 c	3.66	37.76 c	1.54	0.079
	E	76.02 a	3.34	69.84 a	2.07	0.167
	F	72.60 a	4.15	66.48 a	1.59	0.218
	P value	< .0001		< .0001		
Cohesiveness	A	0.47 bc	0.01	0.37 cd	0.05	0.102
	B	0.43 c	0.04	0.30 d	0.04	0.066
	C	0.55 a	0.01	0.48 ab	0.06	0.287
	D	0.47 bc	0.01	0.41 bc	0.03	0.071
	E	0.54 a	0.01	0.53 a	0.02	0.563
	F	0.51 ab	0.03	0.52 a	0.01	0.705
	P value	0.007		0.001		

SE: standard error

<sup>abcde</sup>Data with different letters in each column are significantly different ( $p < 0.05$ )

<sup>1</sup>A: 10% meat protein; B: 8% meat protein; C: 8% meat protein + 0.5% TGase; D: 8% meat protein + 2% pea protein; E: 8% meat protein + 2% pea protein + 0.5% TGase; F: 8% meat protein + (2% pea protein + 0.5% TGase).

Many authors have reported TGase addition produces stronger gels (Pietrasik & Li-Chan, 2002; Sakamoto *et al.* 1995). Motoki and Seguroy (1998) also reported proteins that are not gelled by heating can be gelled by addition of TGase. In this study, at 8% meat protein level, TGase addition increased gel hardness by 75% for the normal group, and 136% for the PSE group (treatment C vs. B). There have been no reports in the literature regarding how TGase would affect the strength of meat gels made from PSE meat. In this study, gels made from TGase-treated PSE meat (8% protein) were



even harder than those made from normal meat alone (53.42 N vs. 37.08 N) (Table 4.2.4).

Pea protein addition significantly improved gel strength (treatment D vs. B), especially for PSE gels. The reason could be attributed to higher total protein content (10% vs. 8%). But samples with 8% meat protein and 2% pea protein (treatment D) still had a softer texture compared to the 10% meat protein control (treatment A), which suggests that pea protein had an inferior gelling function than meat protein under the current conditions even though the total protein content was the same. Shand *et al.* (2007) reported the optimal temperature for pea protein to form a strong gel was 93 °C. Therefore under the heating condition of the current study (80 °C), pea protein may not gel and contribute to gel texture.

Samples containing both pea protein and TGase (treatment E and F) exhibited superior textural properties among all treatments. For example, hardness increased 65% and 85% for normal and PSE groups (treatment E vs. D), respectively. In the presence of TGase, with the same total protein content in the formulation, gel hardness changed from inferior (treatment D vs. A) to superior (treatment E and F vs. A). Shand *et al.* (2008) reported TGase could catalyze the crosslinking of pea proteins and enhance the strength of pea protein gels. It is reasonable to postulate that TGase catalyzed the crosslinking of meat and pea proteins in the gel network and these crosslinkings are strong and hard to break. It is also important to note that after TGase treatment, the strength of the gels made from PSE meat (with pea protein substitution) was brought up to a similar hardness as the gels made from normal meat (without substitution). Likewise, cohesiveness measurement showed a similar trend: TGase alone or combined

with pea protein helped produce more elastic meat gels. For instance, at 8% protein level, TGase addition increased cohesiveness up to 28% and 60% for normal and PSE meat gels (treatment C vs. B), respectively. At 10% total protein level, meat alone (treatment A) and meat plus pea protein isolate (treatment D) had a similar level of cohesiveness, while that of TGase-treated samples (treatment E and F) increased 15% and 43% compared to treatment A and D for normal and PSE meat gels, respectively. This observation suggested that protein crosslinking catalyzed by TGase helped produce a matrix structure in which internal bonds were stronger and connected adjacent particles more closely than TGase-free meat gels. The data also revealed the improvement for gels made from PSE meat was more dramatic than that of gels made from normal meat. There was no significant difference between treatment E and F in terms of TPA results, indicating pre-incubation of pea protein and TGase did not help produce more cohesive gels.

#### 4.2.5 Effect of PPI and TGase on Torsional Gelometry

The results of torsional gelometry showed that meat gels made from normal turkey breast meat had significantly higher shear stress and strain value ( $p < 0.05$ ) than those made from PSE meat with protein content of 10% and 8% (treatment A and B) (Table 4.2.5), indicating gels made from normal meat were harder and more elastic than those made from PSE meat. Compared to study 1, the shear stress value of respective untreated normal and PSE meat gels was much lower in the current study, mainly due to the reduced protein content in the formulation (8-10% vs. 14%). But the respective shear strain value was not much different. These observations are in agreement with the

statement made by Hamann (1988) as cited previously in the literature review: shear stress is strongly influenced by protein concentration, whereas shear strain is affected mainly by protein quality and may be considered an evaluation of protein functionality. In both study 1 and 2, untreated PSE meat gels had a lower shear strain value ( $p < 0.05$ ) than corresponding normal meat gels, indicating PSE meat was inferior to normal meat in terms of protein functionality.

Table 4.2.5 Torsional gelometry analysis of the cooked meat gels with added PPI and TGase

Torsional gelometry	Treatment <sup>1</sup>	Normal (n = 4)		PSE (n = 4)		P value
		Mean	SE	Mean	SE	
Shear stress at failure kPa	A	24.18 c	1.09	15.58 b	1.68	0.005
	B	14.07 d	0.73	11.25 c	1.29	0.016
	C	31.10 b	2.10	27.92 a	2.39	0.357
	D	18.32 d	1.62	16.06 b	1.53	0.350
	E	39.64 a	1.62	32.69 a	3.25	0.104
	F	41.96 a	2.65	31.55 a	4.34	0.087
	P value	< .0001		< .0001		
Shear strain at failure	A	2.01 b	0.04	1.87 b	0.07	0.030
	B	1.99 b	0.06	1.80 b	0.05	0.040
	C	2.34 a	0.08	2.21 a	0.10	0.322
	D	2.11 b	0.08	1.93 b	0.08	0.159
	E	2.38 a	0.04	2.20 a	0.08	0.088
	F	2.36 a	0.06	2.16 a	0.09	0.105
	P value	0.0004		< .0001		

SE: standard error

<sup>abcde</sup>Data with different letters in each column are significantly different ( $p < 0.05$ )

<sup>1</sup>A: 10% meat protein; B: 8% meat protein; C: 8% meat protein + 0.5% TGase; D: 8% meat protein + 2% pea protein; E: 8% meat protein + 2% pea protein + 0.5% TGase; F: 8% meat protein + (2% pea protein + 0.5% TGase).

Pérez-Mateos *et al.* (2002) reported squid muscle gels with added TGase exhibited significantly higher hardness and elasticity than control samples. In the present experiment, at 8% meat protein level (treatment C vs. B), crosslinking of proteins catalyzed by TGase resulted in an increase of 121% and 148% in shear stress, and 18% and 23% in shear strain, for gels prepared from normal and PSE meat, respectively. More importantly, after TGase treatment, 8% protein gels made from PSE meat had much higher shear stress and shear strain values than those made from normal meat. These values were even higher than that seen for 10% protein gels made from normal meat (treatment A).

Pea protein alone (treatment D) did not significantly ( $p > 0.05$ ) improve gel strength for normal meat (treatment D vs. B). In fact substituting meat protein with pea protein produced gels that were softer than the unsubstituted formulation (treatment D vs. A). For PSE meat, pea protein addition only improved gel hardness not elasticity (treatment D vs. B), and pea protein substitution did not improve gel texture when compared to the unsubstituted sample (treatment A). The results showed pea protein alone was not effective in improving gel texture.

Samples containing both pea protein and TGase (treatment E and F) prepared from meat of normal quality exhibited the highest ( $p < 0.05$ ) gel strength. For formulations made with PSE meat, TGase-treated samples (treatment C, E, and F) had significantly higher ( $p < 0.05$ ) gel strength than those without TGase. For example, shear stress increased 64% and 110% for normal and PSE groups, respectively, and shear strain increased 17% and 18% (treatment E vs. A), respectively, with substitution of 2% pea protein and addition of 0.5% TGase. These results showed that with the

combined action of pea protein and TGase, the strength of the gels made from PSE meat became superior to the gels made from normal meat without substitution, which confirmed the previous TPA findings. These results suggested, in the presence of TGase, one could substitute meat protein with pea protein, and expect to improve or at least maintain the same gel strength as the unsubstituted products would have, even for PSE meat.

#### 4.2.6 Effect of TGase Treatment on Protein Crosslinking

The SDS-PAGE profiles of the six treatments are shown in Figure 4.2.1. SDS-PAGE protein banding patterns of normal and PSE meat gels were essentially the same, therefore only a representative image was shown.

Many authors reported TGase can catalyze the formation of glutamyl-lysine bonds in myosin and between myosin and actin (De Backer-Royer *et al.*, 1992), and proteins of lower molecular weight (e.g. tropomyosin and troponin) (Pérez-Mateos *et al.*, 2002) depending on the origin of TGase. The efficiency with which a protein can be utilized as a substrate by TGase is known to be influenced by the amino acid sequence adjacent to the reactive glutamyl residues (Gorman & Folk, 1980).

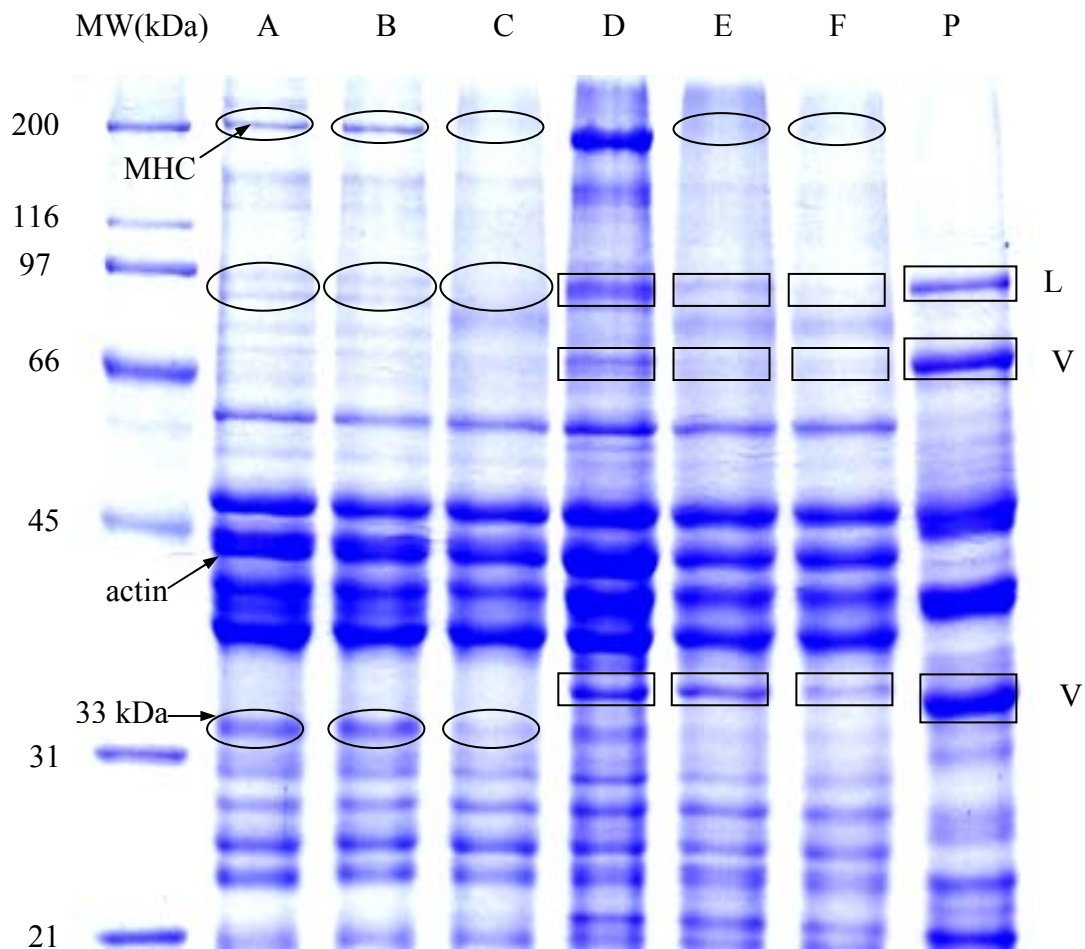


Figure 4.2.1 SDS-PAGE profiles of the cooked meat gels with added PPI and TGase. MW: molecular weight standard. A: 10% meat protein, B: 8% meat protein, C: 8% meat protein + 0.5% TGase, D: 8% meat protein + 2% pea protein, E: 8% meat protein + 2% pea protein + 0.5% TGase, F: 8% meat protein + (2% pea protein + 0.5% TGase). P: 10% pea protein. Approximately 10  $\mu$ g protein was loaded. L: legumin subunits. V: vicilin subunits.

In the present study, some bands (shown in ellipses) in TGase treated samples (lane C, E, and F) disappeared or became lower in density when compared to respective bands in control lanes A and B (Figure 4.2.1). For instance, after TGase treatment (lane C, E, and F), MHC (~ 200 kDa) was completely gone, and a ~ 33 kDa protein band

almost disappeared, but actin (~ 42 kDa) was largely unaffected, which means the currently used TGase had less specificity for actin than for MHC and other affected proteins. The content of some protein fractions from pea protein (shown in rectangles) in TGase treated samples (lane E and F) also decreased when compared to lane P, indicating pea proteins were also crosslinked either within themselves or with meat proteins by TGase. These proteins appeared to be legumin subunits (labeled as L) and vicilin subunits (labeled as V), according to Shand *et al.* (2007). When comparing only lane E and F, it was observed that the bands in rectangles were lighter in lane F than in lane E, probably because the action of pre-incubating pea protein and TGase (treatment F) resulted in more crosslinked proteins which were not extracted into the soluble protein fraction.

Pérez-Mateos *et al.* (2002) concluded fish gels treated with TGase had a greater prevalence of covalent cross-linking where mainly myosin heavy chain (MHC) was involved, and these covalent bonds were so strong that most denaturants could not cleave them. In this experiment, it is possible that the crosslinked MHC or pea proteins were not soluble and subsequently not extracted into the solution, and therefore did not get into the SDS-PAGE system.

It is interesting to note that the addition of pea protein somehow increased the protein extractability of the cooked meat gels as protein bands were shown to be darker in lane D when compared to other lanes, yet the reason is unknown.

#### 4.2.7 Protein Extractability of TGase-Treated Meat Gels

Due to the absence of some bands in the SDS-PAGE profiles shown above, it is reasonable to suspect some proteins have been crosslinked and not extracted into the solution. To verify this speculation, protein extractability of the cooked gels was evaluated for the last two replications of the study.

Cooked meat gels were ground and extracted with 8 M urea and 0.6 M sodium chloride overnight and the protein content was determined by Bradford method (Bradford, 1976) with modifications. The objective of this experiment was to examine the effect of adding pea protein and TGase on protein solubility and not that of meat quality (i.e. normal vs. PSE), so data obtained for meat gels made from normal and PSE turkey meat were pooled together when presenting the results (Table 4.2.6).

Table 4.2.6 Protein extractability\* of the cooked meat gels with added PPI and TGase

Total protein content	Protein content in extract (mg/ml)		
	Normal (n=2)	PSE (n=2)	Pooled (n=4)
A (10% meat protein)	6.12	6.36	6.24 c
B (8% meat protein)	6.15	6.10	6.13 c
C (8% meat protein + TGase)	4.27	4.35	4.31 d
D (8% meat protein + 2% pea protein)	7.95	7.83	7.89 a
E (8% meat protein + 2% pea protein + TGase)	7.07	6.95	7.01 b
F (8% meat protein + 2% pea protein + TGase)	7.09	7.09	7.09 b

\*Extraction buffer: 8 M urea and 0.6 M NaCl. Extraction ratio: 1:10.

<sup>abcd</sup>Data with different letters are significantly different ( $p < 0.05$ )



In Table 4.2.6, treatment A, D, E, and F initially had similar total protein content (10%), but protein solubility of the final product was significantly different. The sample made from meat only (treatments A and B) had the lowest ( $p < 0.05$ ) protein extractability, indicating heat denatured meat protein was more difficult to solubilize than pea protein. It is noted that the sample with added pea protein only (treatment D) had a significantly higher extractability than others (e.g. 7.95 in treatment D vs. 6.15 in treatment B), indicating untreated pea protein was almost all solubilized in the extraction buffer. Protein extractability of TGase-treated samples (treatment E and F) were significantly lower ( $p < 0.05$ ) than that of TGase free sample (treatment D), indicating some pea proteins must have been crosslinked by TGase to each other or with meat proteins or both so that the crosslinked proteins did not solubilize.

Treatment B and C started with the same protein content (8%) from the same origin (meat), yet protein solubility of TGase-treated sample (treatment C) was much lower ( $p < 0.05$ ) than that of the control (treatment B), which suggested TGase must have crosslinked some meat proteins together. Because these crosslinked bonds were so strong, they could not be cleaved by urea and salt. Therefore gels containing TGase had a significantly smaller percentage of soluble protein than those without TGase.

Pérez-Mateos *et al.* (2002) studied effects of TGase on frozen squid muscle gels. They found gels treated with TGase contained a significantly reduced amount of soluble protein than the gels without TGase. The authors concluded that the insoluble protein was highly aggregated by means of covalent bonds, which were not readily cleaved by most denaturants. The formation of covalent bonds has been largely attributed to TGase activity (Pérez-Mateos *et al.*, 2002). This low protein extractability in the present study

confirmed that the absence of some of the protein bands in the SDS-PAGE profiles was the result of unavailability of certain proteins after crosslinking in the loading buffer (Figure 4.2.1).

#### 4.2.8 Effect of PPI and TGase on Rheological Properties

Overall heat-induced rheological changes of meat batters with added pea protein and TGase are displayed in Figure 4.2.2a. The rheograms obtained from normal and PSE samples displayed essentially an identical pattern, and hence, only one set of data is presented. There was no observation of crossover between storage modulus ( $G'$ ) and loss modulus ( $G''$ , not shown in the graph) within the range of temperatures followed, indicating that there was a network structure formed in the sample prior to rheological testing, and the meat batters exhibited a stronger elastic response than viscous response. To better display some details, a different scale was used in Figure 4.2.2b.

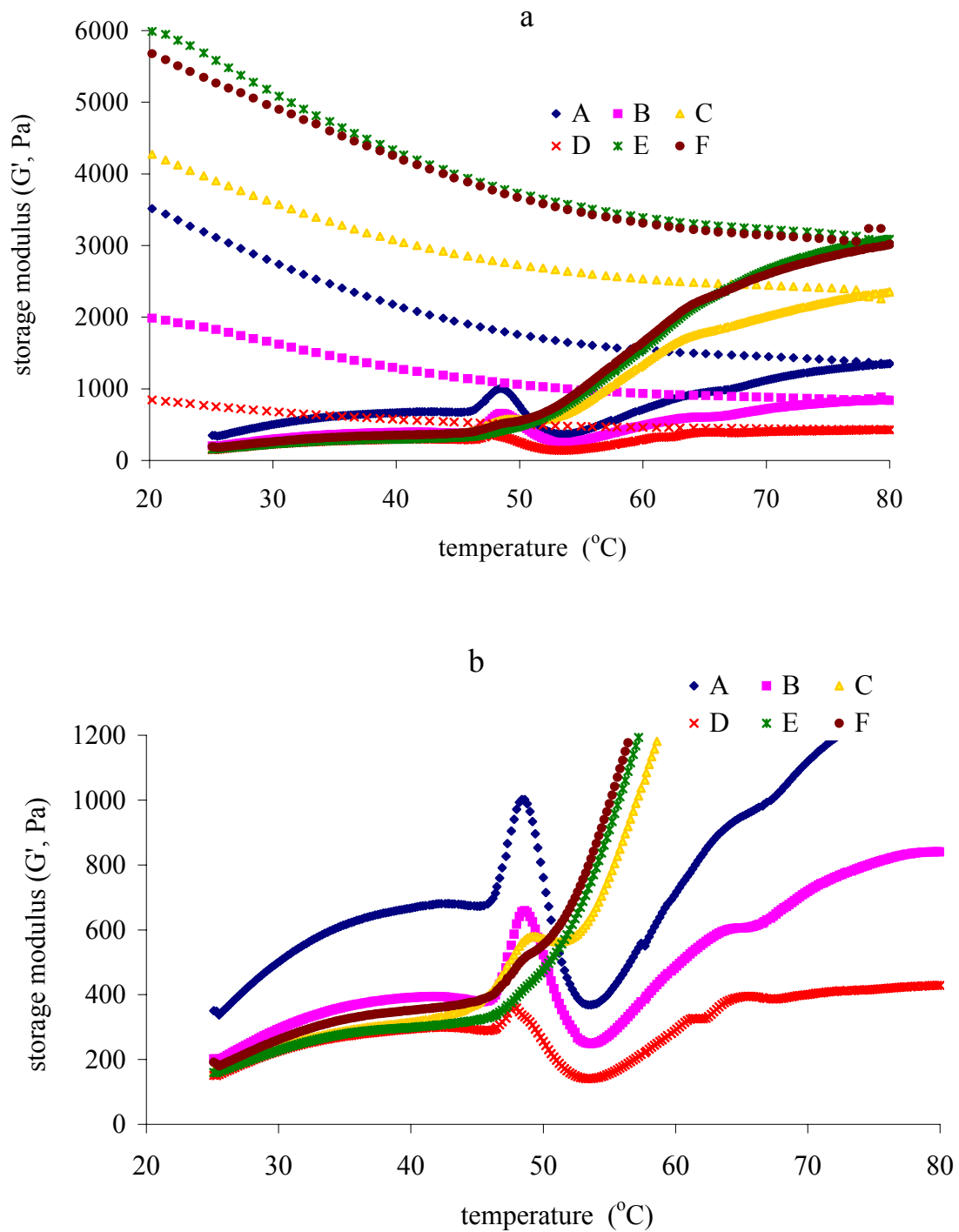


Figure 4.2.2 Representative rheological changes of meat batters during thermal processing. a). Standard  $G'$  plot. b). Enlarged  $G'$  scale region. Heating rate: 1  $^{\circ}\text{C}/\text{min}$ . Cooling rate: 5  $^{\circ}\text{C}/\text{min}$ . Formulations – A: 5% meat protein; B: 4% meat protein; C: 4% meat protein + 0.5%TGase; D: 4% meat protein + 1% pea protein; E: 4% meat protein + 1% pea protein + 0.5% TGase; F: 4% meat protein + (1% pea protein + 0.5% TGase).

Samples containing 5% meat protein (treatment A) showed a higher viscosity at the starting point (25 °C) (see Figure 4.2.2b) than other samples, which may be attributed to the higher percentage of protein extracted out into solution during batter preparation. During heating, all samples first showed a slow and steady increase in viscoelasticity. When heated to ~ 46 °C, all treatments (with or without TGase and pea protein) exhibited a sharp increase in storage modulus ( $G'$ ), likely due to protein aggregation (Xiong & Blanchard, 1994). For samples without TGase, the magnitude of  $G'$ , after reaching a maximum at ~ 48.5 °C, decreased sharply to a minimum at 51 ~ 53.5 °C range, and then sharply increased throughout the rest of the whole heating process. The transitions were not as obvious, however, for TGase-treated samples, and thereafter the  $G'$  increased at a steeper rate than the ones without TGase. During cooling, the  $G'$  values continued to increase for all samples, indicating an elastic gel structure was being formed.

The first transition at ~ 48.5 °C was likely caused by denaturation and aggregation of myosin heads associated with the initial stages of helix-coil transition of the myosin molecule (Fernández *et al.*, 1996). Egelandstad *et al.* (1986) suggested that the  $G'$  decrease was attributed to denaturation of light meromyosin, leading to increased filamental “fluidity”. The  $G'$  increase thereafter probably resulted from formation of more permanent, irreversible myosin filaments or complex. The transition temperatures reported in the literature were slightly different than the present study due to different experimental conditions (e.g. batter composition, ionic strength, and pH).

Samples containing 5% meat protein (treatment A) had a significantly higher peak  $G'$  (~ 1002 Pa) at ~ 48 °C than others. This may be due to the higher percentage of

myofibrillar protein content, mainly myosin. After reaching a minimum at  $\sim 53.5$  °C,  $G'$  started rising again and reached  $\sim 1350$  Pa at 80 °C, and then ended at  $\sim 3560$  Pa after cooling. Samples with 4% meat protein only (treatment B) exhibited an identical curve, but as expected the  $G'$  was always lower because the protein content was lower.

The maximum value of  $G'$  at  $\sim 48.5$  °C was slightly lower for treatment C (4% meat protein + TGase) than for control B (meat only), possibly because TGase already started crosslinking proteins during heating so the structure became more compact and affected further gel network formation (Oakenfull *et al.*, 1997). It is observed that the second increase of  $G'$  for treatment C started at  $\sim 51$  °C versus  $\sim 53.5$  °C for control B, and the increasing slope was steeper, which means probably TGase helped produce more intramolecular crosslinks and these crosslinked proteins had a lower temperature requirement for producing an elastic structure (Ramírez-Suárez & Xiong, 2003a).

Treatment D (4% meat protein + 1% pea protein) showed a substantially reduced  $G'$  peak at  $\sim 48.5$  °C, and did not produce a stronger network than the meat treatment with 4% protein (control B) even though the total protein content was higher. Ramírez-Suárez and Xiong (2002 and 2003b) reported similar findings with other nonmuscle additives. This indicates untreated exogenous proteins (pea protein in this case) had a detrimental effect on muscle protein gelation probably by interfering with protein-protein interaction that is responsible for the formation of elastic structure (Ramírez-Suárez & Xiong, 2003a).

Rheograms obtained for treatment E and F (4% meat protein + 1% pea protein + TGase) basically followed the same pattern. The curves did not have a clear peak, instead they both showed a small shoulder at  $\sim 48$  °C and then increased sharply, ending

up with a much higher  $G'$  value ( $\sim 3000$  Pa) at  $80$  °C than other treatments ( $p < 0.05$ ). After cooling, the  $G'$  reached  $\sim 6,000$  Pa, which is significantly higher than all others ( $p < 0.05$ ). It is possible that TGase catalyzed the crosslinking of meat and pea proteins, changed the gelling pattern during protein gelation and produced more elastic gels. Pre-incubation of pea protein with TGase (treatment F) did not seem to produce a better result for protein gelation compared to treatment E because the  $G'$  value was slightly lower at  $80$  °C. The reason could be that pea proteins were already crosslinked during pre-incubation and could not interact with meat proteins as completely.

Overall rheological changes revealed that TGase can improve gel strength of meat or meat-pea protein systems, pea protein alone could not improve gel strength, and combination of TGase and pea protein can produce the strongest gels under the current conditions. These results basically agreed with the findings obtained from TPA and torsion measurements.

#### 4.2.9 Summary of study 2

Many efforts have been undertaken in an attempt to augment the inferior processing ability of the PSE meat. Transglutaminase (TGase) has been shown to improve textural characteristics of fish surimi and meat products through its ability to catalyze crosslinking of proteins. The purpose of the present study was to determine if the ability of TGase to crosslink proteins could improve textural properties of comminuted PSE turkey meat products. Pea protein isolate was found to be a useful extender to the meat system.

SDS-PAGE patterns showed that 0.5% TGase catalyzed the crosslinking of not only meat proteins but also pea proteins. Dynamic rheological analysis revealed TGase altered the gelation pattern of the meat proteins and produced more elastic meat gels.

Non-muscle proteins generally have been found to be inferior to myofibrillar proteins for functionality in comminuted meat systems. This study indicated that substituting of the meat proteins with pea proteins resulted in meat gels which were less hard and elastic, and had poorer binding capacities, which means pea protein alone could not replace meat proteins in the formulation. Transglutaminase alone significantly improved meat gel texture but released more water as well. The reason could be the crosslinking function of transglutaminase made the meat matrix structure more compact, and expelled more water out of the system. The combination of TGase and pea protein eliminated the adverse effect of pea proteins on muscle protein gelation, produced a rigid mixed protein gel at a reduced myofibrillar protein concentration, and maintained a similar cook yield as in the un-substituted formulation. Hence, in comminuted muscle foods that contain low-cost functional ingredient such as pea proteins, transglutaminase may serve as an excellent agent for producing an adhesive mixed protein gel structure.

Another significant finding of this study is that the crosslinking of meat proteins catalyzed by transglutaminase was shown to be an effective strategy to compensate for the inferior properties of PSE meat in comminuted meat systems, thereby improving the quality of finished products made from PSE meat.

## 5. GENERAL SUMMARY AND CONCLUSION

The occurrence of the PSE condition in the pork industry has been documented for more than 50 years. With the increasing demand for poultry meat, PSE-like quality defects in poultry have also risen in recent years. Extensive research work has demonstrated that PSE development is the result of accelerated glycolysis post mortem triggered by preslaughter stress. It is generally believed that the rapid decline of pH in PSE muscle, combined with high carcass temperature within the first few hours after slaughter, causes the muscle proteins to denature (Fernández *et al.*, 1994). Protein denaturation has a detrimental effect on protein functionality. Therefore not only will the fresh PSE meat have excessive drip loss, but more significantly, the PSE meat has lower value for further processing due to the poor binding properties (Cassens, 2000).

More recently, Rathgeber *et al.* (1999b) found rapid postmortem glycolysis and delay chilling of turkey carcasses caused myosin degradation. Myofibrillar proteins (e.g. myosin) are important in determining water holding capacity of the myofilament lattice and subsequently play a critical role in protein gelation (Offer & Trinick, 1983). Results of the study by Rathgeber *et al.* (1999b) provided insight into the molecular basis for previously reported reductions in meat quality of rapid glycolyzing and delay chilled turkey meat.

The present research evaluated the effects of protein modification on turkey breast meat gelation, especially on PSE meat gelation.



In study 1, different levels of  $\alpha$ -chymotrypsin (EC: 3.4.21.1), a serine proteinase that has the ability to cleave meat proteins, were used to treat turkey breast meat, in the hope to create various degrees of protein degradation as in PSE meat.

As expected, proteolysis of meat proteins with chymotrypsin addition had a significant impact on textural properties of the turkey meat gels. For instance, compared to the control (0 ppm chymotrypsin addition), TPA hardness and cohesiveness of meat gels decreased dramatically under increasing chymotrypsin addition (2.5, 5, and 10 ppm) for both normal and PSE groups. Similarly, torsional gelometry revealed shear stress and shear strain also decreased significantly with increasing chymotrypsin addition. These results demonstrated chymotrypsin treatment (protein degradation) adversely affected textural properties of turkey meat gels.

The SDS-PAGE protein profiles of the cooked meat gels revealed, with chymotrypsin treatment, both normal and PSE turkey meat exhibited various extents of proteolysis. Myofibrillar proteins such as myosin were among the most affected muscle proteins. The changes in rheograms further proved chymotrypsin-treated meat batters exhibited altered transition pattern during gelation and subsequently produced meat gels that were less elastic than untreated samples. Correlation analysis indicated that the amount of intact myosin heavy chain was highly correlated with meat gel textural properties and water-holding capacity, suggesting the completeness of muscle proteins such as MHC is important to gelation.

In study 2, strategies to augment the inferior processing ability of the PSE meat were sought. Transglutaminase (TGase, EC 2.3.2.13), an enzyme that has been proven to have the ability to crosslink various food proteins, was used to improve the poor

binding ability during PSE meat gelation. Meanwhile, pea protein was also added into the formulation to improve textural properties and water holding capacity of meat products.

This study showed TGase crosslinked not only meat proteins but also pea proteins. SDS-PAGE patterns revealed some large proteins, such as myosin heavy chain from meat protein and lipoxygenase from pea protein, were crosslinked by TGase and became insoluble in the extraction solution. Dynamic rheological analysis revealed TGase altered meat protein gelation pattern and produced more elastic meat gels. Texture analysis suggested TGase alone significantly improved meat gel texture but released more water as well. The reason could be that the crosslinking of proteins catalyzed by transglutaminase made the meat matrix structure more compact, and expelled more water out of the system. The combination of TGase and pea protein produced a rigid mixed protein gel at a reduced myofibrillar protein concentration, and maintained a similar cook yield as in the unsubstituted formulation. Hence, in comminuted muscle foods that contain low-cost functional ingredient such as pea proteins, transglutaminase may serve as an excellent agent for producing an adhesive mixed protein gel structure.

As a conclusion, this study suggested protein degradation is closely associated with the inferior processing ability occurred in PSE meat. This study indicated that crosslinking of proteins catalyzed by TGase improved the functionality of PSE meat and improved textural properties of turkey breast meat gels with reduced meat protein content. The combination of pea protein and TGase not only produced the strongest gel texture but also prevented high cook loss resulting by TGase addition alone.

Although textural properties of PSE meat products have been improved through protein modification in this study, the water holding capacity has not seen much improvement. Further research could be focused on exploring the strategies to improve water holding capacity of processed PSE meats.

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