POSTMORTEM CHANGES IN MEAT QUALITY AND MYOFIBRILLAR PROTEIN DEGRADATION IN TURKEY BREAST MUSCLE

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ABSTRACT

The purpose of this research was to evaluate the effect of elevated carcass temperature in combination with rapidly declining pH or postrigor muscle pH on turkey breast meat quality and the extractability and degradation of individual muscle proteins. Two groups of twelve turkey carcasses with similar ultimate pH values were selected based on 15 min postmortem (PM) breast muscle pH (rapid glycolyzing (RG), pH $\leq$ 5.8, and normal glycolyzing (NG), pH >6.0). One side of each carcass was held near 40°C and immersion chilling was delayed until 110 min PM (DC), while the other side was chilled at 20 min PM (IC). Raw breast meat quality was assessed using measurements of colour and protein extractability, while quality characteristics of cooked product were assessed using cook yield and torsion gelometry. The extractability and degradation of 7 specific proteins was monitored using SDS-PAGE and Western blotting. Additionally, holding postrigor turkey breast meat at 40°C was evaluated as a model for mimicking changes due to rapid PM glycolysis.

Sarcoplasmic and myofibrillar protein extractability was reduced for breast samples from RG and DC carcasses as well as for postrigor meat held at 40°C ($P < 0.05$). All colour values ($L^*a^*b^*$) increased for DC ground breast meat. Strain at fracture for breast meat gels was lower for RG samples. Both stress and strain at fracture and cook yield were reduced for DC breast meat gels. The reduction in meat quality measurements was additive for RG samples that were delay chilled.

Densitometry of Western blot analysis revealed that the extractability of glycogen phosphorylase, creatine kinase and M-protein was reduced for RG or DC

samples. Additional bands in SDS-PAGE banding patterns of RG and DC samples were identified as fragments of myosin heavy chain. Myosin degradation in RG/DC samples was as high as 20%. Heating postrigor breast increased myosin degradation, however, differences in the banding pattern of myosin fragments compared to RG and DC samples were observed. Nebulin degradation was more extensive in RG samples than for controls.

The results of these studies provide conclusive evidence that rapid PM glycolysis and delayed chilling have detrimental effects on turkey breast meat quality. Additionally, the association of postmortem protein degradation with meat of reduced quality merits further investigation.

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ATP - Adenosine triphosphate

CAF - Calcium activated factor

DC - Delay chilled

DNTB - 5, 5'-dithiobis(2-nitrobenzoic acid)

HIS - High ionic strength

IC - Immediately chilled

LIS - Low ionic strength

MHC - Myosin heavy chain

MP - Myofibrillar protein

NG - Normal glycolyzing

PM - Postmortem

PSE - Pale soft exudative

RG - Rapid glycolyzing

SDS-PAGE - Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SP - Sarcoplasmic protein

SSS - Standard salt solution

TBS - Tris buffered saline

TTBS - Tris buffered saline with Tween 20

1. INTRODUCTION

Turkey processors have estimated that 5 to 30% of turkey breast meat is unsuitable for manufacture of further processed products (Barbut, 1996a). Breast meat of poor quality is often described as pale with poor water binding capacity and an open coarse texture (Sosnicki and Wilson, 1991; Hollingsworth, 1993; Ferket, 1995; Barbut, 1996a; McCurdy *et al.*, 1996; McKee and Sams, 1997, 1998a). A similar condition in pork, known as pale, soft, exudative (PSE), has a long history compared to this relatively new problem in the turkey industry (Hollingsworth, 1993; Ferket, 1995). Therefore, current hypotheses regarding poor quality pale turkey breast meat are often based on what has been previously established with PSE pork.

Pork described as PSE has a pale unstable colour, high drip loss, and reduced protein extractability (Bendall and Wismer-Pedersen, 1962; Warner *et al.*, 1997). Reduced protein extractability in PSE pork has been attributed to denaturation of both sarcoplasmic and myofibrillar proteins, particularly myosin (Stabursvik *et al.*, 1984; Offer, 1991). It is believed that protein denaturation occurs during times when the muscle pH is low and carcass temperatures are high (Offer, 1991), which can occur if postmortem glycolysis is unusually rapid (Boles *et al.*, 1992; Louis *et al.*, 1993) or carcass cooling is slow (Bendall and Wismer-Pedersen, 1962; Offer, 1991; Fernandez *et al.*, 1994). The development of PSE pork is often associated with exposure to preslaughter stress, which can trigger an accelerated breakdown of

muscle glycogen in pig muscle and result in very low muscle pH early postmortem while the carcass temperature is still high (Offer, 1991; Boles *et al.*, 1992; Louis *et al.*, 1993).

Factors that influence the incidence of turkey breast with PSE-like characteristics are strikingly similar to conditions established for the development of PSE pork. The occurrence of PSE-like turkey breast peaks during the warm summer months of the year (Hollingsworth, 1993) and can be increased experimentally by exposure to conditions of heat-stress (McKee and Sams, 1997). There is an indication that rapid postmortem glycolysis is associated with the development of PSE-like characteristics (Pietrzak *et al.*, 1997; McKee and Sams, 1997, 1998a). Accelerated postmortem glycolysis in turkey breast meat has been observed by monitoring early postmortem breast muscle pH (Ma and Addis, 1973; Pietrzak *et al.*, 1997). Pietrzak *et al.* (1997) reported that breast meat from turkey carcasses with a breast muscle pH < 5.8 at 20 min postmortem was paler and had lower protein extractability than breast meat from carcasses with a pH > 5.8.

To evaluate the effect of elevated temperatures on meat quality when the muscle pH is low, some investigators have taken postrigor muscle and returned it to physiological temperatures. Fischer *et al.* (1979) and Sakata *et al.* (1981, 1983) have used the holding of postrigor pork at 40°C as a model for the development of PSE-like characteristics in meat.

There are several advantages to using a postrigor heating model for evaluating turkey meat quality. One advantage is that comparisons can be made between PSE and normal meat from the same animal. Additionally, it is much easier

to make comparisons of postrigor meat exposed to different combinations of temperatures than selecting meat that has been naturally exposed to the temperature and pH combinations of interest during rigor development. However, to provide useful information this model should mimic changes to meat quality that occur during rigor development.

There are currently very few published investigations on the effects of rapid postmortem glycolysis on turkey breast meat quality (Pietrzak *et al.*, 1997; McKee and Sams, 1997, 1998a). These studies generally do not address the influence of potential differences in the postrigor pH of muscle on breast meat quality. The postrigor pH can greatly influence properties such as colour (Swatland, 1995), protein extractability, cook yield, and stress and strain at fracture of cooked meat gels (Daum-Thunberg *et al.*, 1992). Therefore, it is important to distinguish between changes to breast meat quality caused by rapid postmortem glycolysis and changes caused by differences in final muscle pH.

In order to establish the influence of the rate of postmortem glycolysis on turkey breast meat quality, comparisons should be made between normal and rapid glycolyzing carcasses. Carcasses representing each extreme can be selected based on early postmortem muscle pH measurements (Pietrzak *et al.*, 1997). The rate of postmortem glycolysis can be increased by reducing the rate of carcass temperature decline (Greaser, 1986). A reduced rate of carcass chilling may magnify changes to breast meat quality as well as magnifying any alterations to individual proteins that may be related to reduced breast meat quality. Therefore, any subtle differences in meat quality or alterations to proteins should be more easy to detect.

OBJECTIVES

1) To evaluate holding postrigor turkey breast muscle at physiological temperatures as a model for PSE-like breast meat.

2) To evaluate the influence of the rate of postmortem breast muscle pH decline in combination with modified carcass chilling regimes without the influence of differences in postrigor pH on colour and quality of turkey breast meat.

3) To evaluate the influence of the rate of postmortem breast muscle pH decline and delayed chilling of carcasses on the extractability and/or degradation of individual proteins.

2. LITERATURE REVIEW

2.1 TURKEY BREAST MEAT QUALITY

Lifestyle changes in North America have dramatically increased the demand for foods that are easy to prepare in a short time. The response of the poultry industry to this situation has been to increase the production of further processed and fully cooked products (Ferket, 1995). In the United States in particular, approximately 70% of turkey was marketed as a whole carcass product in the 1980's (Ferket, 1995) with 30% as further processed or value added. By 1995, the situation was reversed with approximately 70% of turkey sold as further processed or value added product.

Coincidentally, as the market for further processed turkey increased, a serious problem with the quality of the breast meat became apparent to processors for as many as 30% of turkey carcasses (Barbut, 1996a). Poor quality turkey breast meat is typically paler, has poor water holding capacity, and a coarse open structure (Sosnicki and Wilson, 1991; Hollingsworth, 1993; Ferket, 1995; Barbut, 1996a; McCurdy *et al.*, 1996; McKee and Sams, 1997, 1998a). This problem is especially noticeable in further processed turkey breast products such as cooked turkey breast rolls. Inclusion of poor quality turkey breast meat in a cooked roll results in poor colour uniformity and a product that crumbles apart when thinly sliced (Ferket,

1995). Additionally, there is often a problem with excessive purge of fluid in the package following cooking of the turkey roll (Ferket, 1995; Barbut 1996a). This unsightly accumulation of fluid requires that the product be rebagged resulting in an economic loss from both reduced product yield and additional handling (Barbut, 1996a).

The assessment of meat quality for fabrication and processing of acceptable products requires attention to numerous functional roles (Briskey and Kauffman, 1978). These functional roles include, water-binding, emulsification, gel formation, adhesion, fat binding, tenderness, texture, and juiciness of the cooked product (Briskey and Kauffman, 1978). It is important to emphasize that these aspects of meat quality are influenced by the animal's age, nutrition, genetic development, and postmortem metabolism (Briskey and Kauffman, 1978). Postmortem metabolism during the conversion of muscle to meat is also influenced by many factors such as muscle fiber type, muscle glycogen reserves, antemortem stress, and rate of carcass cooling (Greaser, 1986). Thus the mechanisms for development of poor quality turkey breast meat may be very complex.

Much of the knowledge regarding the cause of poor quality pale turkey breast meat has been derived by applying what has been already established for a similar condition in porcine muscle, namely PSE pork (Hollingsworth, 1993; Ferket, 1995; Pietrzak *et al.*, 1997). Antemortem stress initiates an accelerated rate of postmortem glycolysis in both turkeys (Mckee and Sams, 1997) and pigs (Offer, 1991) causing muscle pH to be low before substantial carcass cooling has occurred. The rate of postmortem metabolism in skeletal muscle of both species seems to be

the most important factor involved in the development of PSE pork and PSE-like turkey breast meat (Offer, 1991; Hollingsworth, 1993; McKee and Sams, 1997; Pietrzak *et al.*, 1997; Sosnicki *et al.*, 1998). Therefore, it is important to understand the specific changes that occur during the postmortem conversion of muscle to meat.

2.1.1 CONVERSION OF MUSCLE TO MEAT

Many of the reactions involved in the normal function of skeletal muscle in a living animal influence the postmortem changes in muscle when it becomes meat. The primary function of skeletal muscle in higher organisms is the locomotion of the individual. The myofibril or contractile element is the predominant organelle of skeletal muscle and is responsible for the development of muscle tension (Bechtel, 1986; Pearson and Young, 1989). When a nervous impulse reaches the motor end plate of a muscle cell, the sarcolemma depolarizes and the signal travels along the T-tubules until Ca^{2+} is released into the sarcoplasm of the cell from the sarcoplasmic reticulum (Bechtel, 1986). The Ca^{2+} binds to troponin C initiating the sliding of the thick and thin filaments past each other, which in turn changes the length of the sarcomere (Pearson and Young, 1989). Energy for contraction is supplied in the form of adenosine triphosphate (ATP), which is hydrolyzed by the ATPase located in the head of the myosin molecule of the thick filament (Pearson and Young, 1989). In order for muscle relaxation to occur, ATP is required for pumping the excess Ca^{2+} from the sarcoplasm back into the sarcoplasmic reticulum (Pearson and Young, 1989).

When a turkey dies, a complex process of metabolic, physical and structural alterations to muscle occurs that result in the conversion of muscle to meat (Greaser, 1986). Although death of the bird occurs in a matter of minutes, its muscle cells continue to metabolize and respond for hours after respiratory cessation and brain death. The stoppage of blood flow ensures there are no new sources of energy or oxygen delivered to the muscle (Greaser, 1986). The muscle cell attempts to maintain ATP levels needed for a number of metabolic processes. However, myosin ATPase and other ATPases soon deplete ATP reserves. The creatine phosphate reserves are the first source of energy used to replenish ATP (Greaser, 1986). After creatine phosphate is depleted, the main source of energy comes from hydrolysis of glycogen stores (Greaser, 1986). After death of the bird, elimination of metabolic wastes via the circulatory system can no longer occur. Therefore, the muscle pH declines as a result of lactic acid accumulation. The size of skeletal muscle glycogen stores is one of the main influences on the extent of muscle pH decline (Pearson and Young, 1989). As pH declines, the ability of the sarcoplasmic reticulum to efficiently sequester Ca^{2+} from the sarcoplasm is reduced (Greaser *et al.*, 1969) increasing the stimulus for contraction. Additionally, permanent cross-bridges begin to form because the ATP necessary for the dissociation of actin and myosin becomes increasingly limited and the muscle begins to stiffen. The onset of rigor mortis or rigor is defined as the time when ATP levels decrease to < 1.0 μmol/ g of muscle (Pearson and Young, 1989).

For pigs which are genetically predisposed to stress, production of lactic acid can occur very rapidly due to a defect located in the ryanodine receptor. The

ryanodine receptor is a homotetrameric protein that bridges the transverse tubule and the terminal cisternae membranes of the sarcoplasmic reticulum acting as a pathway for calcium release (Sosnicki, 1993). Pigs carrying this gene can not resequester Ca^{2+} at an appropriate rate to allow for relaxation following initiation of muscle contraction (Fujii *et al.*, 1991; Louis *et al.*, 1993). Muscle contractions during times of stress are very severe and increase the potential for rapid accumulation of lactic acid early postmortem (Fujii *et al.*, 1991; Louis *et al.*, 1993). Although there is no current evidence for the existence of the same defect in the ryanodine receptor of turkeys, work by Wang *et al.* (1999) suggests that Ca^{2+} regulation in the muscle cells of turkeys currently commercially available is impaired compared to historical lines of turkeys.

2.1.1.1 COMMERCIAL TURKEY SLAUGHTER

The conversion of muscle to meat is initiated by the slaughter process, which consists of several stages in a commercial poultry processing facility. Once turkeys reach a desirable market weight they are transported to the processing facility. The birds are denied access to feed for several hours prior to transport to reduce the volume of gut contents in order to minimize fecal contamination of the carcass (Wood, 1989; Sams and Mills, 1993).

Upon arrival at the plant, birds are manually hung on shackles attached to an overhead conveyor (Mountney and Parkhurst, 1995). The birds' heads are guided through a trough of saline, that has been electrified. This stuns the birds prior to the

severing of the jugular veins on one side of the neck (Mountney and Parkhurst, 1995). Alternatively birds can be exposed to atmospheres low in oxygen and/or high in carbon dioxide as a means of immobilizing the birds prior to ensanguination (Sams, 1999). Electric stunning of poultry reduces the rate of postmortem glycolysis compared to gas stunning (Kang and Sams, 1999). However, proponents of gas stunning cite the fact that gas-stunned poultry have fewer broken bones and hemorrhages than electrically-stunned birds (Kang and Sams, 1999).

Following ensanguination the birds travel through a bleed out area that allows several minutes of time for blood drainage. The birds are then immersed in heated water (59 to 60°C) for 2.5 to 2.8 min to facilitate mechanical removal of feathers from the carcass. The carcasses pass through a series of mechanical pickers equipped with rubber fingers that rub the feathers off. Following feather picking the feet are removed at the hock joint. The carcasses are deposited onto a conveyer where they are manually rehung onto the evisceration line where the viscera are removed in several steps. Evisceration of chicken broilers and smaller birds is normally an automated process, however, evisceration of turkeys is often performed manually by a number of people each with their own specific duties.

Following evisceration, carcasses are immersed in cold water (0 to 1°C) or transferred to a cooler where forced cold air reduces the carcass temperature (Mountney and Parhurst, 1995). Chicken broiler carcasses are chilled to below 4°C within 1.5 h by immersion chilling. However, it may take 3 to 6 h of immersion chilling to reduce the carcass temperature to 4°C for large turkeys. Air chilling is even a slower process (Sams, 1999). Carcasses are sometimes aged at refrigeration

temperatures prior to meat removal from the bones (Sams, 1999). If the meat is removed during rigor development the contraction of the muscle in the absence of skeletal support results in severe shortening of the muscle and ultimately tough meat (Sams, 1999). Once the meat is removed from the carcass it can be used in a great number of further processed products.

2.1.2 MUSCLE PROTEINS IMPORTANT TO PROTEIN FUNCTIONALITY

The functional properties of meat proteins have a great influence on the quality of processed meat products. In the context of meat processing, protein functionality is usually described in terms of hydration, surface properties, binding, and rheological behavior (Xiong, 1994). Protein functionality measurements of particular importance to meat scientists include water holding capacity, protein extractability, and meat gel strength (Wang and Smith, 1994; Xiong, 1994) because these measurements correlate well with the yield, texture and palatability of processed meat products (Lopez-Bote *et al.*, 1989; Camou and Sebranek, 1991; Xiong, 1994).

The functional properties of proteins are ultimately determined by the three dimensional arrangement of the protein (Stabursvik *et al.*, 1984; Xiong, 1994). Proteins are essentially a linear chain of covalently bonded amino acids (primary structure) (Lehninger, 1982a) arranged in space by interactions between the side chains of adjacent amino acids (secondary structure) (Lehninger, 1982b) and interactions between amino acids of adjacent polypeptides within the same chain

(tertiary structure) or between chains (quaternary structure) (Lehninger, 1982c). During the process of denaturation the characteristic three-dimensional arrangement of the polypeptide chains are disrupted by unfolding into random and irregular conformations without damage to the primary structure of the protein (Lehninger, 1982c). Exposure to extremes in pH and/or elevated temperatures are common methods for protein denaturation. Denaturation can alter both the biological function of the protein (Lehninger, 1982a) and its functional properties in a food system (Stabursvik *et al.*, 1984; Xiong, 1994).

The vast differences in structure among proteins of skeletal muscle ensures that some proteins contribute more to the functional properties than others. The salt-soluble myofibrillar proteins are primarily responsible for the important functional properties of meat (Morrissey *et al.*, 1987; Pearson and Young, 1989; Wang and Smith, 1994) while the water-soluble sarcoplasmic proteins play a minor role (Pearson and Young, 1989). Myofibrillar proteins account for approximately 50-55% of the total protein in skeletal muscle (Yates and Greaser, 1983).

Myosin is the most abundant and most important protein with respect to functional properties important in meat processing (Samejima *et al.*, 1984; Sharp and Offer, 1992; Wang and Smith, 1994; Xiong, 1994). Myosin comprises ~45% of the total myofibrillar component of skeletal muscle proteins and is easily the most extensively studied myofibrillar protein (Morrissey *et al.*, 1987). The myosin molecule consists of six subunits, two myosin heavy chains (MHC), two alkali light chains and two other light chains that received their name from the fact that they are released from the myosin molecule when treated with 5,5'-dithiobis (2-nitrobenzoic

acid) (DTNB) (Samejima *et al.*, 1984; Pearson and Young, 1989). The four light chain subunits are located in the globular head region of the myosin molecule. The alkali light chains are essential for ATPase activity while the DTNB-light chains regulate ATPase activity (Pearson and Young, 1989). The myosin light chains do not contribute to the gelation potential of myosin at normal postmortem muscle pH values (Samejima *et al.*, 1984). The ability of meat products to form heat-induced protein gels is due mainly to MHC subunit of myosin (Sharp and Offer, 1992; Samejima *et al.*, 1984).

Proteolytic cleavage of MHC with trypsin or chymotrypsin leaves fragments named light meromyosin (LMM) and heavy meromyosin (HMM). The LMM fragment contains the tail or rod portion of the myosin molecule and is almost entirely α helical in structure (Pearson and Young, 1989). Strong ionic interactions between the rod regions of adjacent myosin molecules in the LMM regions are responsible for the aggregation of myosin into thick filaments (Pearson and Young, 1989). Proteolysis of HMM with papain produces the S1 and S2 fragments. The active site for ATPase activity is located near two sulfhydryl groups in the S1 fragment from the head region of HMM (Pearson and Young, 1989).

Actin is the second most common myofibrillar protein making up about 22% of the total myofibrillar proteins (Pearson and Young, 1989). The thin filaments of the myofibril are composed of actin. The contribution to protein functionality from actin is dependent largely upon its interaction with myosin (Samejima *et al.*, 1984). In postrigor muscle, a large portion of actin is bound to myosin and is often referred to as actomyosin (Pearson and Young, 1989). The myosin-to-actin ratio and the

proportion of free to bound actin influence the gelation properties of myosin. The myosin-to-actin ratio varies for different muscle types. Dudziak *et al.* (1988) reported that the myosin-to-actin weight ratios for postrigor turkey breast was 3.8:1 compared to 6.9:1 for turkey thigh. Dissociation of actin from myosin by addition of pyrophosphate increases the amount of free actin and reduces the elasticity of heat-set meat gels (Wang and Smith, 1995).

The exact sequence of events in the gelation of myofibrillar proteins vary with species, heating conditions, buffer systems and for reasons still unknown (Wang and Smith, 1994). In general, during the process of heat-set gelation, the rod portion of myosin unfolds weakening the interaction between myosin molecules. This is followed by aggregation of the myosin head regions (Wang and Smith, 1994). Interaction between actin and myosin stabilizes myosin during the formation of heat-set meat gels by delaying the initial unfolding of myosin (Wang and Smith, 1995).

2.1.2.1 THERMAL STABILITY OF SKELETAL MUSCLE MYOSIN

Not only is myosin the most abundant and important protein with regard to protein functionality (Acton *et al.*, 1983), but it is one of the least thermally stable of the proteins found in muscle (Wright and Wilding, 1984). Differential scanning calorimetry (DSC) studies have determined that the lowest thermal transition in meat is due to the initiation of myosin denaturation (Quinn *et al.*, 1980; Stabursvik *et al.*, 1984; Wright and Wilding, 1984; Xiong *et al.*, 1987; Xiong, 1992). The first thermal transition temperature has been associated with the S1 section of HMM (Bertazzon

and Tsong, 1989) and proteolytic fragments containing a region known as the hinge region (Sutoh *et al.*, 1978). Conformational changes at the hinge regions allows the molecule to have the flexibility needed to permit changes in lateral distance between thick and thin filaments during contraction (Huxley, 1969; Harrington, 1971; Goodno and Swenson, 1975).

Depending on pH, fiber type, ionic strength, and state of rigor development, the onset temperature for the first transition of myosin can be variable (Xiong *et al.*, 1987; Xiong, 1992; Egelandsdal *et al.*, 1994). Egelandsdal *et al.* (1994) reported that the onset of the first thermal transition for calorimetry measurements of bovine myosin occurred at a lower temperature for white fibers compared to red fibers when the pH was either 7.0 or 5.5. This situation was reversed at pH 6.0, where myosin from red fibers had a lower onset temperature for the first transition than myosin from white fibers. White muscle fibers are predominantly from fast twitch muscle that is more developed for anaerobic metabolism whereas red muscle fibers are from more aerobic slow twitch muscle (Pearson and Young, 1989). Turkey breast is composed exclusively of white fibers (Wiskus *et al.*, 1976) and postmortem muscle pH is often at pH 5.8 or less (Barbut, 1996a), indicating turkey breast myosin may be more easily denatured than myosin from livestock species with predominantly red muscle tissue.

2.1.2.2 OTHER STRUCTURAL MYOFIBRILLAR PROTEINS

There are numerous other myofibrillar proteins present in much lower quantities than myosin that are important to the structure of the myofibril (Pearson and Young, 1989). It has been suggested that part of the difference in protein extractability between meat composed of fast white fibers compared to slow red fibers may be due to different degrees of structural hindrance conferred by different isoforms of structural proteins (Xiong, 1994). Some of the structural proteins include desmin, myomesin, M-protein, titin and nebulin. Desmin is a structural protein that binds adjacent muscle fibers together at the Z-band and is highly susceptible to proteolysis (Pearson and Young, 1989). M-protein and myomesin are structural proteins of the M-line that also interact with myosin keeping the thick filaments aligned at the center of the sarcomere (Grove *et al.*, 1985). Titin is the largest muscle protein constituting 8 to 10% of the myofibrillar proteins. It extends from the Z-line at the end of the sarcomere to the M-line in the center (Tan *et al.*, 1993). Although the direct contribution of titin to functional properties is unknown, its role as a scaffold for the positional stability of myosin (Tan *et al.*, 1993; Robson *et al.*, 1997) could be significant with respect to overall functionality. The contribution to protein functionality of another large structural protein, nebulin, is largely unknown. Nebulin interacts with actin keeping it in register in the sarcomere (Pearson and Young, 1989; Robson *et al.*, 1997).

2.1.3 INFLUENCE OF MUSCLE pH AND TEMPERATURE ON PROTEIN FUNCTIONALITY AND MEAT COLOUR

The rate of pH decline in postmortem muscle and the pH that muscle attains postrigor are of major importance to meat quality with respect to the functionality of the proteins and the colour of meat (Offer, 1991; Wang and Smith, 1994; Xiong, 1994). In addition, the rate of postmortem carcass temperature decline can influence protein functionality and meat colour (Fernandez *et al.*, 1994; Pospiech *et al.*, 1997). Changes in the functionality of meat proteins are often associated with changes in protein structure and/or protein denaturation. The influence of the rate of postmortem pH and temperature decline as well as postrigor pH are discussed in the following sections.

2.1.3.1 RATE OF POSTMORTEM pH DECLINE

Pale, soft, exudative pork is often used as an example of how rapid postmortem glycolysis can affect meat colour and reduce protein functionality. Kauffman *et al.* (1993) reported that approximately one fourth of pork carcasses in the United States could be classified as PSE. In a study by Camou and Sebranek (1991), the pH of muscle from normal glycolyzing pork was approximately 6.2 at 45 min postmortem, but the average pH of PSE muscle was 5.6 at 45 min postmortem.

Rapid postmortem glycolysis can also occur in other species of domestic animals commonly slaughtered for meat. Even for cattle, generally regarded as the slowest of the domestic species to reach postrigor pH values, there are occasionally individuals which can be categorized as fast or rapid glycolyzing (O'Halloran *et al.*,

1997). However, the effect of rapid postmortem glycolysis on bovine meat quality is minimal and is the subject of few reports compared to other species. Avian species also demonstrate variability in the rate of postmortem glycolysis. Vanderstoep and Richards (1974) found that some turkey carcasses from a flock of minimally stressed birds exhibited abnormally rapid rates of postmortem glycolysis based on depletion of ATP. Ma and Addis (1973) reported that when turkeys were allowed to struggle during slaughter glycolysis was complete in some birds as early as 5 min postmortem. The possibility of a rapid rate of postmortem glycolysis in turkey breast meat indicates potential for the occurrence of a condition similar to PSE pork.

Wismer-Pederson (1959) determined that pigs stressed prior to slaughter were more prone to developing PSE meat than minimally stressed animals. Heat-stress and struggling prior to slaughter can trigger acceleration of postmortem glycolysis in both pork (Mitchell and Heffron, 1982) and turkeys (Froning *et al.*, 1978; McKee and Sams, 1997). The problem of PSE-like turkey breast meat quality is more prevalent in the warm summer months (Hollingsworth, 1993; Ferket, 1995; Barbut, 1996a) and has been referred to as "the summer yield problem" (Sosnicki *et al.*, 1998). In a study by McKee and Sams (1997) 17-wk old turkey toms were exposed to environmental temperatures of 32/38°C (night/day) compared to controls at 16/24°C. When the birds were slaughtered at 21 wk of age, the heat-stressed birds had significantly lower breast muscle pH at 15 min postmortem (approximately 5.85 vs 5.95) compared to the controls. Measurements of ATP levels early postmortem revealed more rapid depletion of ATP in the breast muscle from heat-stressed birds compared to controls. However, the breast muscle pH at 24 h

postmortem was also significantly lower for heat-stressed turkeys which would contribute to any observed differences in meat quality between the groups of carcasses.

2.1.3.2 POSTRIGOR MUSCLE pH

The postrigor or ultimate pH of meat influences most measurements of protein functionality as well as the colour of meat (Daum-Thunberg *et al.*, 1992; Xiong, 1994; Ferket, 1995; Swatland, 1995). When the ultimate pH is equal to the isoelectric point of a protein there is no net charge between the charged groups of the protein (Pearson, 1986). The isoelectric point of the major proteins of skeletal muscle myofibrils is near 5.0. At this pH the repulsion between individual myofibrils is minimum (Pearson and Young, 1989). Repulsion at pH values above and below the isoelectric point increases the distance between myofibrils increasing the space for accommodating water. The increase in net charge increases the attraction between water and proteins of the myofibril (Pearson and Young, 1989).

The ultimate pH of meat can vary widely, depending mainly on the glycolytic potential of the meat prior to onset of rigor mortis. In a study by Barbut (1997) the ultimate pH of breast meat from a population of mature turkey hens ranged from 5.68 to 6.64. Muscles predominately composed of white fast twitch fibers generally have greater quantities of stored glycogen than muscles with mainly red or slow twitch fibers (Pearson and Young, 1989). Therefore, the ultimate pH of turkey breast meat (pH 5.8 - 6.0) is generally lower than thigh meat (pH 6.4) (Richardson

and Jones, 1987; Daum-Thunberg *et al.*, 1992; Xiong *et al.*, 1993). However, glycogen stores in skeletal muscle may be depleted by fasting or due to exhaustive exercise (Sayre *et al.*, 1963; Wittman *et al.*, 1994). The length of time turkeys are denied access to feed prior to slaughter can be quite variable (Wood, 1989; Sams and Mills, 1993). Low or depleted glycogen reserves will result in meat with higher ultimate pH values compared to birds with full glycogen reserves prior to slaughter (Wittman *et al.*, 1994). Therefore, manipulation of preslaughter practices such as feed withdrawal time may influence final turkey breast meat quality.

2.1.3.3 INFLUENCE OF MUSCLE pH ON WATER HOLDING PROPERTIES

Water is a major constituent of muscle, accounting for about 75% of the weight of fresh muscle tissue (Wismer-Pedersen, 1978; Morrissey *et al.*, 1987). Water influences the juiciness, tenderness, colour, and taste of meat (Wismer-Pedersen, 1978). Both water-protein and protein-protein interactions are involved in holding water in muscle tissue. Protein-protein interactions determine the size of spaces in which water molecules are held in the protein network (Wismer-Pedersen, 1978). About 90 to 95% of water in muscle is referred to as bulk phase water which is immobilized in the spaces between myofibrils (Morrissey *et al.*, 1987; Fennema, 1996). The remaining water is referred to as bound water which is associated with the hydrophilic groups of proteins (Wismer-Pedersen, 1978).

Hamm (1986) defined the water holding capacity of meat as the ability of meat to hold its own water and added water during the application of an external

force such as pressing or centrifugation. However, other authors have provided more specific definitions that differentiate between water retention of raw and cooked meat (Richardson and Jones, 1987). Jauregui *et al.* (1981) defined the water binding potential of meat as the ability of raw meat to hold added water in the presence of or absence of salt under an external force. While drip loss is the fluid lost from raw meat without the application of an external force, other than gravity (Savage *et al.*, 1990; Northcutt *et al.*, 1994), expressible moisture is the amount of fluid expressed from a cooked meat system under an external force (Jauregui *et al.*, 1981). Cook yield of meat and meat products is a simple measurement of the weight lost during cooking expressed as a percentage of the weight prior to cooking or as percentage of the original weight of the meat prior to addition of water (Shand, 1999). This simple measurement of yield is a valuable tool for meat processors when determining the profit margin for production of a product.

The pH of muscle can greatly influence the water holding capacity of meat proteins (Offer, 1991; Wang and Smith, 1994; Xiong, 1994). When the ultimate pH is equal to the isoelectric point, the water holding capacity of meat is at its lowest (Pearson, 1986). The net charge of the amino acids of the muscle proteins increases at pH values above and below the isoelectric point increasing attraction between water and proteins of the muscle cell. Increasing the net charge also increases distances between myofibrils due to repulsion, resulting in more space for accommodating bulk water (Pearson and Young, 1989).

Daum-Thunberg *et al.* (1992) investigated the effect of pH on comminuted turkey breast and thigh meat, and found that cook yield and water holding capacity

increased as pH increased. Barbut (1993, 1996a) also found that the ultimate pH of turkey breast meat was positively correlated to cook yield and water holding capacity. Both McKee and Sams (1997) and Pietrzak *et al.* (1997) monitored the rate of postmortem pH decline in turkey breast meat in order to evaluate changes to water holding properties. In the study by McKee and Sams (1997), breast meat from heat-stressed turkeys exhibited a more rapid postmortem decline in pH. The breast meat from heat-stressed turkeys had increased drip loss (0.44% vs 0.06%) and cook loss (24.6% vs 19.1%) compared to breast meat from unstressed birds. However, the ultimate pH of breast meat from heat-stressed turkeys was significantly lower (~5.85 vs ~5.96) than from unstressed birds, which also may have influenced water holding. Pietrzak *et al.* (1997) selected two groups of turkey carcasses based on extremes in breast muscle pH values at 20 min postmortem. They measured excess water holding capacity and cook yield of breast meat from carcasses with breast muscle pH values < 5.8 at 20 min postmortem compared to a group of carcasses with pH values > 5.8. The rapid glycolyzing breast meat had a lower water holding capacity (84.9% vs 112.4%) and cook yield (107.1% vs 126.1%) than the meat with a pH > 5.8 at 20 min. There was no attempt made in this study to compare carcasses of similar ultimate pH values to determine the effect of the rate of pH decline alone.

2.1.3.4 INFLUENCE OF MUSCLE pH ON PROTEIN EXTRACTABILITY

Extraction of proteins in solutions of high ionic strength is an important physiochemical process in the manufacture of processed meat products (Xiong,

1994). Extraction of proteins commercially is usually a result of comminuation and mixing of meat in the presence of salt (Xiong, 1994). Myofibrillar proteins extracted from the interior of muscle serve as an excellent adhesive, binding surfaces of meat pieces together in restructured meat products (Asghar *et al.*, 1985).

Protein extractability measurements for meat are generally inexpensive and rapid. However, not only are protein extractability measurements, like other protein functionality measurements, affected by muscle fiber type, pH, temperature and rate of postmortem metabolism (Foegeding, 1987; Richardson and Jones, 1987; Xiong, 1994); reported values are also subject to variations due to equipment and methodology (Patel and Fry, 1987; Lan *et al.*, 1993). Differences in protein extractability methods are as numerous as the investigators who perform them (Weinberg and Rose, 1960; Chaudhry *et al.*, 1969; Maxon and Marion, 1969; Richardson and Jones, 1987; Boles *et al.*, 1992; Lan *et al.*, 1993; van Laack *et al.*, 1993; Xiong *et al.*. 1993). Generally, measurement of protein extractability depends on a physical means for separation of the insoluble proteins from those extracted into solution. Centrifugation and filtration, or combinations of both, are the most commonly used systems for separation of extractable proteins from the remaining proteins (Patel and Fry, 1987). However, there is no universally accepted method for determination of protein extractability or water holding capacity of meat (Xiong, 1994). Therefore, absolute values for protein extractability measurements have less meaning than relative differences for comparisons among treatments.

One of the most prominent changes to meat quality in PSE pork is the reduction in protein extractability (Bendall and Wismer-Pedersen, 1962; Fischer *et*

al., 1979; Offer, 1991; Boles *et al.*, 1992; van Laack *et al.*, 1993). Using a centrifugation-based method, Boles *et al.* (1992) reported reductions in the extractability of both sarcoplasmic and myofibrillar proteins from the muscle of pigs genetically predisposed to stress. In a study by van Laack *et al.* (1993), a filtration-based method was used to determine that the combined extractability of sarcoplasmic and myofibrillar proteins from PSE pork was reduced to 68% of normal meat. Bendall and Wismer-Pedersen (1962) attributed reduced myofibrillar protein extractability to the precipitation of denatured sarcoplasmic proteins on the myofibrils. They observed that some sarcoplasmic proteins were not washed out of myofibrils from PSE pork muscle. Pietrzak *et al.* (1997) used immunofluorescent staining to determine that the sarcoplasmic protein, glycogen phosphorylase, was bound to the Z-line region of washed myofibrils from turkey breast with a pH < 5.8 at 20 min postmortem.

2.1.3.5 INFLUENCE OF MUSCLE pH ON MEAT COLOUR

The colour of meat and meat products is often the basis for acceptance or rejection because physical appearance is the main characteristic for evaluation of quality available to the consumer (Cross *et al.*, 1986). Colour measurement studies have been used extensively to determine the incidence of PSE pork (Warner *et al.*, 1997; Cheah *et al.*, 1998). The paleness of PSE pork is thought to be due to increased reflectance of light caused by precipitation of denatured sarcoplasmic proteins on myofibrils (Pearson and Young, 1989; Swatland, 1995).

The use of colour measurements has been touted as a rapid, nondestructive method to sort PSE-like turkey breast from darker normal breast meat in commercial slaughter facilities following deboning (Barbut, 1993, 1996a, 1997; McCurdy *et al.*, 1996). Increased Hunter L* values were reported by Barbut (1993, 1996a, 1997) and McCurdy *et al.* (1996) for paler turkey breast meat samples. The L* values for turkey breast were negatively correlated with water holding capacity, cook yield and ultimate pH (Barbut, 1993, 1997) indicating that the paler meat was indeed of reduced quality. However, in a report by Swatland (1995), meat with lower pH had increased reflectance of incident light causing the meat to appear paler. No measurement of early postmortem breast muscle pH was made in the studies by Barbut (1993, 1996a, 1997), therefore, it is not possible to determine if the reported increased paleness and the reduction in meat quality was a result of accelerated postmortem glycolysis or lower ultimate muscle pH or both.

2.1.3.6 INFLUENCE OF MUSCLE pH ON THERMALLY SET MEAT GELS

In thermally processed meat products, myofibrillar proteins are responsible for the formation of a network that binds meat pieces together, stabilizes fat and water, and gives the product a desirable texture (Hamann, 1988; Xiong, 1993). Sensory analysis can be used to evaluate the texture of processed meat products, or alternatively, instruments that measure the stress and strain at fracture can be used to obtain objective measurements that mimic fracture during mastication (Hamann, 1988). Camou and Sebranek (1991) observed that rapid postmortem pH decline of

pork carcasses affected subsequent meat gelation characteristics. They found that when gels were made from PSE pork the gel strength was 45% of gels made from normal pork with similar ultimate pH values.

In the studies by Barbut (1993, 1996a, 1997) and McCurdy *et al.* (1996) the strength of gels made from turkey breast was measured by a penetration test consisting of plunging a probe into cooked gels. Colour measurements were used to determine the incidence of pale turkey breast in these studies. They found the strength of gels was negatively correlated to L* values indicating the paler breast meat produced weaker gels. However, L* values were also negatively correlated to ultimate pH of the breast meat.

Northcutt (1994) performed torsion tests on gels made from breast meat from a group of pale turkey breasts and a group of dark turkey breasts. Torsion testing is a type of large-strain testing consisting of twisting a uniform cylinder of meat and measuring of the stress and strain at the point of fracture (Hamann, 1983). Northcutt (1994) did not observe any differences in gel strength between pale and dark breast meat when ultimate pH was held constant.

Contradictory results of Barbut (1993, 1996a, 1997) and Northcutt (1994) emphasize the influence of ultimate pH on turkey breast meat functional properties. Selection of turkey breast meat based on paleness did not give an indication of how the rate of postmortem glycolysis affected meat quality. However, what these results did show is that differences in ultimate pH should be taken into account when evaluating the effects of the rate of postmortem glycolysis on meat quality.

2.1.3.7 INFLUENCE OF CARCASS TEMPERATURE ON MEAT QUALITY

Not only is meat quality influenced by muscle pH, but extremes in carcass temperatures have the potential to seriously alter the quality of meat harvested from slaughtered animals (Pearson and Young, 1989; Offer, 1991; Fernandez *et al.*, 1994). If the temperature of prerigor meat drops to low levels, a phenomenon known as cold shortening can occur (Pearson and Young, 1989). The Ca^{2+} binding capacity of the sarcoplasmic reticulum and mitochondria of muscle cells is reduced at temperatures below 10°C, resulting in increased contractions and excessive shortening of sarcomeres in muscle fibers (Pearson, 1986). This excessive shortening of muscle fibers is responsible for increased toughness of meat. However, cold shortening is restricted almost exclusively to muscle composed of red fibers. The sarcoplasmic reticulum of white fibers is more developed than in red fibers and not affected to the same degree by low temperature (Pearson, 1986). Therefore, cold shortening primarily affects species that contain a greater proportion of meat composed of red fibers such as cattle and sheep and to a lesser extent pigs.

If the temperature of prerigor meat remains high the metabolic processes that occur during the development of rigor are accelerated (Pearson and Young, 1989). High temperature conditioning, as it is known in the beef industry, can be used to accelerate glycolysis in order to reduce the time required for aging prior to removal of meat from the carcass (Pospiech *et al.*, 1997). For pigs, with a much shorter time course of rigor than cattle, high temperature conditioning can lead to the development of meat with PSE characteristics (Bendall and Wismer-Pedersen, 1962; Offer, 1991; Pospiech *et al.*, 1997). Chaudhry *et al.* (1969) found that myofibrillar

protein solubility was reduced when prerigor rabbit muscle was held at 37°C. Penny (1977) reported that prerigor pork held at 37°C had increased drip loss than controls held at 10°C. The drip loss was 1-2% for samples held at 10°C and increased to 10-14% for samples held at 37°C. Fernandez *et al.* (1994) held pork at 10 or 35°C during rigor development and found a reduction in the extractability of water- and salt-soluble proteins for samples held at 35°C. This effect was diminished in carcasses with higher ultimate pH values, indicating there is an important relationship between pH and temperature of meat with regard to protein quality.

Investigators have reported changes to meat quality when chicken is held at elevated temperatures during rigor development. Bilgili *et al.* (1989) evaluated the effect of postmortem temperature on sarcomere length, cooking loss, and tenderness of broiler breast meat during the aging process. They were concerned that the early entry into immersion chillers may cause cold shortening. For 4 h following evisceration the carcasses were held at 0, 14, 28, or 41°C and then chilled to 4°C by immersion in ice water for 1 h. Results indicated the greatest amount of sarcomere shortening was for samples held at 0°C indicating that chicken may be susceptible to cold shortening. The 41°C treatment resulted in the highest shear values and cook loss for cooked breast meat of all the treatments.

McKee and Sams (1998a) conducted a study to determine the effect of postmortem carcass temperature on the rate of postmortem glycolysis in turkey breast. They immersed turkey carcasses in water with temperatures of 40, 20, or 0°C for the first 4 h immediately after evisceration. The rate of postmortem glycolysis was determined by measuring glycogen levels, pH and ratio of

inosine:adenosine (R-value) at 15 min, 30 min, 1 h, 2 h, and 4 h postmortem. At 4 h postmortem the breast muscle was removed, bagged and aged an additional 20 h on ice. Aged breasts were cooked and shear force measurements were made. Postmortem glycolysis was the most rapid in the 40°C treatment as indicated by the fastest decline in pH and fastest depletion of glycogen in the first 4 h. The conversion of adenosine to inosine was significantly greater by 15 min postmortem in the samples held at 40°C and persisted though the 4 h period. Breast meat from carcasses held at 40°C was paler by 4 h postmortem than breast meat held at 0 or 20°C. Breast meat from carcasses held at 40°C had the greatest drip loss after 24 h, the highest shear values, and greatest cook loss of all the treatments. This research indicates that postmortem glycolysis can be accelerated in turkey breast muscle by holding a carcass at 40°C early postmortem. However, part of the differences observed in cook loss and shear force may have been due to differences in ultimate pH values for the different treatments.

The size of turkey carcasses can influence the postmortem cooling rate of the *Pectoralis major*. Currently, genetic selection for increased muscle size of the modern turkey has resulted in a carcasses that were 27% heavier at 18 wk of age in 1995 compared to turkey toms from 1985 (Sell, 1995). Therefore, adequate cooling rates for larger carcasses with current processing procedures and chilling equipment may be becoming more difficult to achieve. Inadequate cooling rates would be especially problematic during times when there are stoppages in the processing line due to mechanical breakdown. Additionally, time at the rehang station, where

carcasses are manually returned to the shackles of the slaughter line following hock removal, can be variable and may increase the time before initiation of chilling.

2.2 PROTEOLYTIC DEGRADTION OF MUSCLE PROTEINS

The reduced functional properties of PSE pork and rapid glycolyzing turkey breast have been attributed to non-specific denaturation of muscle proteins. However, there is evidence that postmortem degradation of some proteins is altered in PSE pork and rapid glycolyzing turkey breast and may be responsible for a part of the reduced protein functionality. Boles *et al.* (1992) found a reduction in the rate of postmortem degradation of the cytoskeletal proteins titin and nebulin in PSE pork. These large proteins serve as scaffolds for the alignment of other myofibrillar proteins and are found to degrade more slowly in meat of reduced tenderness (Robson *et al.*, 1997). A reduction in postmortem degradation of titin in turkey breast meat was reported in an abstract by Pospiech *et al.* (1992) for turkeys with low pH early postmortem. It has been suggested that a reduction in the postmortem degradation of these large scaffold proteins may reduce the extractability of other proteins of the myofibril (Boles *et al.*, 1992).

Other than the abstract by Pospiech *et al.* (1992) on titin degradation ,the degradation of proteins in rapid glycolyzing turkey breast have not received much attention. The results of the report by Pospiech *et al.* (1992) on titin degradation were based on subjective evaluation of fluorescence of titin antibodies attached to myofibrils prepared from rapid glycolyzing and normal turkey breast meat. The

authors stated that weak titin staining of myofibrils occurred in 22% of the total samples of myofibrils from normal glycolyzing breast meat compared to 2% for rapid glycolyzing turkeys. A more effective way to monitor degradation of proteins is to use a combination of sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) and Western blotting (Bandman and Zdanis, 1988). The proteins are separated by SDS-PAGE and then fragments are identified using antibodies specific to the protein of interest.

2.2.1 CALPAINS AND CATHEPSINS

In skeletal muscle there are several groups of proteases that are involved in maintenance of muscle integrity and mass (Goll *et al.*, 1983). These endogenous proteolytic enzymes are often classified by their mode of activation as well as where they are located within the muscle cell (Pearson and Young, 1989). Two main groups of proteases important in postmortem meat are calpains and cathepsins (Etherington *et al.*, 1987). Calpains are cytosolic proteases activated by elevated levels of Ca^{2+} and cathepsins are lysosomal enzymes active at low pH (Goll *et al.*, 1983).

Calpains were first referred to as calcium activated factor (CAF) by Busch *et al.* (1972) and were later given the more common name in use today. Two forms of calpain are present in skeletal muscle, one activated by millimolar levels of Ca^{2+} and the other requiring micromolar levels of Ca^{2+} (Dayton *et al.*, 1981). Not only is the activity of calpains controlled by Ca^{2+} levels, but an inhibitor known as calpastatin

also influences the activity of calpains (Johari *et al.*, 1993). Changes that occur in a muscle postmortem can have an effect on calpain activity. As the pH of muscle declines, the sarcoplasmic reticulum loses its ability to accumulate Ca^{2+} from the sarcoplasm (Greaser *et al.*, 1969). An increase in Ca^{2+} levels promotes calpain activity, however, this is modulated in postrigor muscle because the optimum pH for calpain activity is between 6.5 and 8.0 (Goll *et al.*, 1983).

The majority of investigations involving calpain activity have attempted to explain the role of calpains in postmortem tenderization of meat (Johnson *et al.*, 1990; Dransfield, 1994; Taylor *et al.*, 1995; Huff-Lonergan *et al.*, 1996). The degradation of Z-line proteins was initially thought to be the key in postmortem tenderization (Nagainis *et al.*, 1983; Goll *et al.*, 1983). However, more recent studies indicate that the degradation of the large cytoskeletal and costameric proteins may be more important than Z-line degradation in postmortem tenderization (Taylor *et al.*, 1995).

There are a number of proteolytic lysosomal enzymes known as cathepsins (Pearson and Young, 1989). Cathepsins are most active at low pH (4.0 to 6.0) and temperatures near 37°C (Bird and Carter, 1980). This is similar to the conditions within the lysosome of a living muscle cell (Pearson and Young, 1989). Cathepsin activity is greatly reduced at low temperatures and inhibited by cystatin, an inhibitor located in the sarcoplasm (Etherington *et al.*, 1987). Some of the proteins degraded by cathepsins include troponin T (Noda *et al.*, 1981), actin (Hirao *et al.*, 1984), and myosin (Zeece and Katoh, 1989; Whipple and Koohmaraie, 1991; Matsuishi *et al.*, 1992; Jiang *et al.*, 1996).

A number of myofibrillar proteins are degraded by cathepsins, however, the importance of cathepsin degradation in relation to meat quality has not been established (Johnson *et al.*, 1990). Wang and Xiong (1998) demonstrated that increased cathepsin activity in bovine cardiac muscle reduced the strength of surimi gels made from cardiac myofibrils. Therefore, there may be a relationship between degradation of proteins and protein functionality.

There is an indication that the activity of both calpains and cathepsins are affected by the rate of postmortem glycolysis in bovine muscles. In a study by O'Halloran *et al.* (1997), rapid postmortem glycolysis increased the release of cathepsin B and L from lysosomes and increased the activity of calpains. The decreased degradation of titin and nebulin in PSE pork reported by Boles *et al.* (1992) suggests that changes to protease activity due to the rate of postmortem glycolysis may be different for different animal species.

2.3 SUMMARY

The problem of poor quality pale turkey breast meat has received significant attention in the 1990's (Pospiech *et al.*, 1992; Barbut, 1993, 1996a; McCurdy *et al.*, 1996; Pietrzak *et al.*, 1997; Barbut, 1997; McKee and Sams, 1997, 1998a, 1998b; Sosnicki *et al.*, 1998). The postmortem changes that occur during the conversion of muscle to meat appear to be key to understanding the development of poor quality turkey breast meat. Postmortem rigor development in turkey breast meat is more rapid than most domestic animal species and can be accelerated even further by

postmortem struggling (Froning *et al.*, 1978) or antemortem heat-stress (McKee and Sams, 1997). Rapid postmortem pH decline in pork muscle prior to substantial carcass cooling, results in conditions favorable for denaturation of muscle proteins that cause PSE pork (Offer, 1991). Many investigators believe that PSE-like turkey breast is also the result of unusually rapid postmortem rigor development.

To conduct investigations into the meat quality of PSE-like turkey breast, the breast meat with PSE-like characteristics needs to be induced and/or selected from a population of turkey carcasses. One method used to produce PSE-like meat with pork muscle was to hold postrigor muscle at elevated temperatures (Fischer *et al.*, 1979; Sakata *et al.*, 1981, 1983). The advantages of using such a model for producing PSE-like turkey breast are numerous. Accurate adjustment of the temperature and pH of meat could provide meaningful information regarding the effect of combinations of temperature and pH on meat quality.

There are two main approaches used for selection of PSE-like turkey breast from turkey carcasses. Based on the knowledge that PSE pork is significantly paler than normal pork, some investigators have elected to select pale breast meat following deboning and compared this to normal or dark breast meat (Barbut, 1993, 1996a; McCurdy *et al.*, 1996; Barbut, 1997). This procedure may provide valuable information to turkey processors, however, in the absence of early postmortem pH measurements this evaluation may simply be a comparison of the quality of muscle with a low ultimate pH to a group with high ultimate pH. It has been well established that ultimate pH of muscle affects the lightness of meat as well as many quality measurements (Pearson and Young, 1989).

Another approach to evaluate the reported poor quality breast meat problem is to actually select turkey carcasses based on differences in early postmortem breast muscle pH (Pospiech *et al.*, 1992; Pietrzak *et al.*, 1997; Sosnicki *et al.*, 1998). The impact of the rate of postmortem glycolysis on turkey breast quality can be established by this approach providing carcasses selected have similar ultimate pH values. The rate of postmortem glycolysis can be further accelerated by reducing the rate of carcass temperature decline (Greaser, 1986). A combination of selection based on early postmortem muscle pH values and reducing the rate of carcass chilling could be used to magnify the changes in breast meat quality.

Whether the postrigor heating model is employed or breast meat is selected based on early postmortem muscle pH measurements, methods to measure differences in meat quality need to be used. Protein functionality measurements have been used as tools to determine the influence of the rate of rigor development on meat quality for both pork (Lopez-Bote *et al.*, 1989; Camou and Sebranek, 1991; Offer, 1991; Xiong, 1994) and turkey breast meat (Pietrzak *et al.*, 1997; Sosnicki *et al.*, 1998). Typical protein functionality measurements include protein extractability, cook yield, expressible moisture and cooked gel strength measurements.

Muscle proteins important to the structure and function of myofibrils may be susceptible to denaturation and/or enzymatic degradation. Alterations to the structure of muscle proteins can be determined by changes in extractability of these proteins (Fischer *et al.*, 1979; Pietrzak *et al.*, 1997; van Laack *et al.*, 1993) and/or by observation of proteolytic fragments by SDS-PAGE in combination with Western blotting (Huff-Lonergan *et al.*, 1996). Changes in the extractability (Pietrzak *et al.*,

1997) and/or degradation of muscle proteins (Boles *et al.*, 1992) in relation to changes in meat quality could provide meaningful information regarding the molecular basis for changes in protein functionality.

3. MATERIALS AND METHODS

3.1 POSTRIGOR HEATING TRIALS

Several preliminary studies were conducted to evaluate the effect of holding postrigor turkey breast samples at several temperatures on protein extractability measurements. Initially postrigor turkey breast samples were held at temperatures of ~ 25, 30, 35, 40 and 45°C for durations ranging from 15 to 120 min. Additionally, pH adjustments were made to evaluate the influence of extremes in muscle pH. However, protein extractability results were extremely variable in these preliminary studies making interpretation of results very difficult. It was suspected that a large part of the source of the variation was from the method used to measure protein extractability, which was based on filtration of the samples. The main objective of the postrigor heating trials was to evaluate holding postrigor turkey breast at physiological temperatures as a model for PSE-like breast meat. A secondary objective of one of the following experiments was to evaluate two protein extractability methods, one based on centrifugation and one based on filtration for protein separation. The combinations of exposure time and holding temperature were limited to 30 and 120 min and 0 and 40°C.

3.1.1 TURKEY BREAST MUSCLE SAMPLE PREPARATION

Hybrid tom turkeys were raised to 116 days of age at the University of Saskatchewan Poultry Facility and processed at Lillydale Poultry (Wynyard, SK). Five carcasses were chosen at random following immersion chilling, packed in ice, transported to the University of Saskatchewan, and stored at ~ 4°C. Two days postmortem the *Pectoralis major* was removed, cut into ~100 g samples, vacuum packaged, and frozen at ~ -40°C. Samples were removed from ~ -40°C storage and tempered at ~ -1°C for 12 h prior to the experiment. Visible fat and connective tissue were trimmed from tempered samples. Then samples were ground through a 20-mm plate followed by a 3-mm plate (with chilled equipment).

A 40.0 g (± 0.5 g) sample of ground meat was homogenized (Polytron PT 10/35, Brinkman Instruments, Mississauga, ON) in 200 mL of deionized water (~ 4°C) containing 1mM NaN_3 as an antimicrobial agent (Yamamoto *et al.*, 1979) and 2mM EDTA to inhibit calpain activity (Uytterhaegen *et al.*, 1994). The meat was homogenized for six 10 s intervals and placed on ice. A sample of the homogenate was warmed to ~ 22°C and the pH measured (Accumet 915 pH meter, Fisher Scientific, Ottawa, ON). Kjeldahl nitrogen (Official method 981.10, AOAC, 1990) of the homogenate was determined and multiplied by 6.25 to obtain a measure for total protein of the samples. Six gram (± 0.03g) samples of homogenate were removed from this pool and added to 50 mL centrifuge tubes, capped to minimize evaporation, and put on ice.

Thirty-two meat homogenate samples were assigned at random to one of 16 possible treatments, with two samples per treatment. These treatments were a result

of all possible combinations of two temperatures (0 or 40), two exposure times (30 or 120 min), two protein extraction solutions (low or high ionic strength), and two separation methods (centrifugation or filtration). Protein in the filtrate or supernatant was measured both at 30 min and 48 h after the extraction of proteins.

3.1.2 POSTRIGOR HEATING

Samples were placed in an ice water bath or 40.0 $\pm$ 0.2°C covered water bath for 30 or 120 min. After heat treatment, the samples were placed in ice water for 15 min.

3.1.3 PROTEIN EXTRACTABILITY

To each homogenate sample, 20 mL of chilled (either LIS or HIS) buffer (~ 4°C) was added. A low ionic strength phosphate buffer (LIS; 0.06 M potassium phosphate buffer, 1 mM NaN_3 2 mM EDTA, pH 7.3) was added to extract the sarcoplasmic proteins or a high ionic strength buffer (HIS; 0.06 M potassium phosphate buffer, 0.69 M KCl , 1 mM NaN_3, 2 mM EDTA, pH 7.3) was added to extract both the sarcoplasmic and myofibrillar proteins (Xiong *et al.*, 1993). With the initial 5 mL of water added to each gram of meat taken into account (from preparation step), the extraction solutions had a similar ionic strength to buffers used by Boles *et al.*, (1992), van Laack *et al.*, (1993) and Xiong *et al.*, (1993) to extract meat proteins. Following the addition of extraction buffer the samples were homogenized for 10 s and placed in an ice water bath for 30 min.

3.1.3.1 FILTRATION METHOD

The vacuum filtration procedure for protein extractability in this investigation was a modification of the method described by van Laack *et al.* (1993). To increase the rate of filtration, a 40 μm pore size pre-filter (VWR grade 417, VWR Scientific, Mississauga, ON) was placed on top of a Whatman No. 1 filter paper (Whatman Laboratory Division, Maidstone, Kent, UK). A vacuum generated by a water aspiration unit facilitated filtration. The protein in the filtrate was determined using the Biuret procedure at 540 nm (Gornall *et al.*, 1949). Bovine serum albumin was used as the protein standard. Protein measurements were made within 30 min of filtration and again after 2 days of refrigerated storage (~ 4°C) to determine if proteins extracted remained in solution.

3.1.3.2 CENTRIFUGATION METHOD

The samples assigned to the centrifugation treatment were centrifuged at 17,500 x *g* for 15 min at ~ 2°C; a modification of the procedure described by Xiong *et al.* (1993). The protein in the supernatant was measured as described for the filtered samples.

3.1.4 PROTEIN EXTRACTABILITY CALCULATIONS

The protein extracted was determined by multiplying the volume of filtrate or supernatant by the number of milligrams of protein per milliliter. This figure was

divided by the total amount of protein in the sample then multiplied by 100 to give a percentage of the total protein.

3.1.5 SELECTION OF RAPID GLYCOLYZING TURKEY CARCASSES

To determine if observed differences due to postrigor heating were influenced by the rate of postmortem glycolysis, additional turkey carcasses were selected based on the differences in early postmortem breast muscle pH. Four commercially raised tom turkey carcasses weighing approximately 6.5 kg were selected from the processing line of Lillydale Poultry (Wynyard, SK) at 15 min postmortem. Two carcasses with a 15 min postmortem breast muscle pH < 5.80 were classified as rapid glycolyzing (RG) and carcasses with a 15 min postmortem pH > 6.00 were classified as normal glycolyzing (NG). Breast muscle pH was measured using a model HI9025 portable pH meter (Hanna Instruments, Sigma Chemical Company, St. Louis, MO) equipped with a spear tip electrode (Mettler Toledo, Wilmington, MA). The electrode was inserted into the *Pectoralis major* at the edge of the feather tract directly ventral to the wing joint.

The selected carcasses were immersion chilled in ice water for 60 min followed by packing with ice in insulated boxes. The boxes containing the carcasses were transported to the University of Saskatchewan, where they were stored at approximately -1°C.

Forty-eight hours postmortem, 2 rectangular samples measuring 1 x 1 x 5 cm were removed from the *Pectoralis major* of each carcass. Each sample was vacuum

sealed in a bag. One sample from each carcass was placed in a ice water slush and the other was immersed in a 40.0 $\pm$ 0.2°C water bath for 90 min and then cooled in ice water for 1 h. Washed myofibril samples were prepared for SDS-PAGE and Western blotting as described in section 3.3.1.2.

3.2 QUALITY OF RAPID GLYCOLYZING AND DELAY CHILLED TURKEY BREAST

3.2.1 CARCASS SELECTION AND PREPARATION

Thirty tom turkey carcasses weighing between 10.5 and 12 kg were selected from the commercial processing lines of Lillydale Poultry (Edmonton, AB and Wynyard, SK) at 15 min postmortem over five slaughter dates. These carcasses were different than those used in the postrigor heating trials. Carcasses with a 15 min postmortem breast muscle pH $\leq$ 5.80 were classified as RG and carcasses with a 15 min postmortem pH > 6.00 were classified as NG (Table 3.1). Breast muscle pH was measured in triplicate using a model HI9025 portable pH meter (Hanna Instruments, Sigma Chemical Company, St. Louis, MO) equipped with a spear tip electrode (Mettler Toledo, Wilmington, MA). The electrode was inserted into the *Pectoralis major* at the edge of the feather tract directly ventral to the wing joint.

Table 3.1 - The number of carcasses selected at 15 min postmortem and retained at 36 h postmortem and carcasses used for breast meat quality measurements

Slaughter day	Carcasses[3] selected 15 min postmortem	Carcasses selected 36 h postmortem	Breast meat quality measurements
1	4 NG[1]	2 NG	2 NG
	2 RG[2]	2 RG	2 RG
2	3 NG	2 NG	2 NG
	2 RG	2 RG	2 RG
3	2 NG	2 NG	2 NG
	3 RG	2 RG	2 RG
4	2 NG	2 NG	2 NG
	4 RG	2 RG	2 RG
5	4 NG	4 NG	
	4 RG	4 RG	
Total	15 NG	12 NG	8 NG
	15 RG	12 RG	8 RG

[1]NG = Normal glycolyzing.
[2]RG = Rapid glycolyzing.
[3]All carcasses were split prior to chilling with one half immediately chilled and the other half delay chilled.

3.2.2 DELAY CHILL PROCEDURE

Following rate of rigor classification, carcasses were split in half through the spine and center of the keel with a hand-held meat saw to obtain left and right sides. Any damage to muscle tissue was noted for later reference. At 20 min postmortem, one carcass half was chosen at random and placed in a static ice water bath. The other half was placed in a Styrofoam box containing a filled hot water bottle (2 L at 40 ± 3°C) until 110 min postmortem when it was transferred to the ice water bath.

All carcass halves were chilled for a total of 60 min in the ice water followed by packing with ice in insulated boxes.

3.2.3 CARCASS TEMPERATURE MONITORING

The boxes containing the carcasses were then transferred to a cooler (0 ± 2°C), stored overnight, and transported to the University of Saskatchewan, where they were stored at ~ -1°C. Temperature of the approximate geometric center of the *Pectoralis major* was monitored for one carcass half from each chilling treatment on three collection days. The temperature was measured every minute starting at 15 min postmortem for 18 to 24 h with temperature tracking devices (TempTale3™, Sensitech Inc., Beverly, MA; ICON™ Data Logger, DeltaTRAK Inc., Pleasanton, CA).

3.2.4 BREAST MUSCLE SAMPLE PREPARATION

At 18 to 24 h postmortem, a 2 to 3 g sample of *Pectoralis major* was removed from an area next to the anterior point of the keel from all carcass halves. These samples were frozen at ~ -40°C and later used for the preparation of washed myofibril samples. Additionally, on the last slaughter date similar samples were removed from four RG and four NG carcasses at 15 min and at 3 h postmortem. These samples were immediately frozen in liquid nitrogen until arrival at the University where they were transferred to ~ -40°C storage. The 3 h samples were

used for preparation of washed myofibrils and the 15 min samples were used for pH measurements.

At 18 to 24 h postmortem the *Pectoralis major* was removed from each carcass half. The exterior surface of each breast sample was removed to a depth of 1 cm to avoid inclusion of tissue damaged by exposure to extreme temperatures during the scalding procedure. Areas of tissue damage from the process of splitting the carcass in half were also trimmed. The breast fillets were placed into plastic bags and stored overnight at ~ -1°C. At 36 h postmortem, the breast samples were ground through a 20-mm plate followed by a 3-mm plate (~ 4°C equipment), mixed, and held at ~ 4°C for < 1 h prior to use.

3.2.5 RAW BREAST MUSCLE MEASUREMENTS

3.2.5.1 MUSCLE pH

The pH of the samples frozen in liquid nitrogen at 15 min postmortem was determined according to the iodoacetate method (Jeacocke, 1977) as modified by Northcutt *et al.* (1998) to confirm the classification of carcasses as RG or NG. At 36 h postmortem, the ultimate pH of breast samples was determined using ~ 20 g of tissue blended for 1 min in 80 mL deionized water. The pH of the slurry was determined with a standard Accumet pH electrode (Accumet, Fisher Scientific, Nepean, ON). At this point, RG carcasses were paired with NG carcasses with similar breast muscle ultimate pH and retained for further experimentation. A total of 24 (12 NG and 12 RG) carcasses were retained for further analysis (Table 3.1).

Sixteen (8 NG and 8 RG) of these carcasses from the first four slaughter dates were used for breast meat quality measurements (Table 3.1).

3.2.5.2 GROUND BREAST MEAT COLOUR

A ground breast meat sample from each carcass half (16 IC and 16 DC) collected on the first four slaughter dates (8 NG and 8 RG) was firmly pressed into a plastic disposable petri dish (diameter 5.3 cm, depth ~ 1 cm) and stored in the dark at ~ 4°C. Fifty min after grinding the colour of the sample was determined in duplicate with a HunterLab MiniScan XE™ (Hunter Associates Laboratories Inc., Reston, VA). The instrument was set to measure CIE $L^*a^*b^*$ using illuminant A and 10° standard observer. The instrument was calibrated using black and white tiles. A pink tile was used to monitor consistency from day to day. The duplicate measurement was taken after the sample was turned 90° from the first measurement.

3.2.5.3 PROTEIN EXTRACTABILITY

The extractability of sarcoplasmic and myofibrillar proteins was determined using procedures modified from Xiong *et al.* (1993) and Boles *et al.* (1994). One-gram samples of ground breast meat were homogenized (Polytron PT 10/35 Brinkman Instruments, Mississauga, ON) in 20 mL of low ionic strength (LIS) buffer (0.05 M potassium phosphate, 1 mM NaN_3, 2 mM EDTA, pH 7.3, ~ 4°C) for 10 s and placed on ice for 30 min. These samples were centrifuged at 17,500 x *g* for 15 min at ~ 2°C. The supernatant was retained as the sarcoplasmic protein extract and

the pellet was resuspended in an additional 20 mL of LIS buffer, homogenized and centrifuged as in the previous step. The supernatant was discarded and the procedure was repeated with 20 mL of high ionic strength (HIS) buffer (0.55 M KCl, 0.05 M potassium phosphate, 1 mM NaN_3, 2 mM EDTA, pH 7.3, ~ 4°C). Following centrifugation, supernatant was removed and retained as the myofibrillar protein extract.

The amount of sarcoplasmic and myofibrillar protein extracted was determined by multiplying the volume of the extraction buffer by the number of milligrams of protein per milliliter. This figure was divided by the original amount of protein in the sample, then multiplied by 100 to give a percentage of the original protein. Protein concentration of all supernatants was determined using the Biuret procedure (Gornall, 1949) with bovine serum albumin as the standard. Original protein was calculated following Kjeldahl nitrogen determination (Official method 981.10, AOAC, 1990) of each ground meat sample and multiplying by 6.25.

The first LIS extract and the HIS extract were retained for SDS-PAGE analysis. In addition to samples from the carcasses collected on the first four slaughter dates, the protein extractability procedure was performed on the carcass halves from the last slaughter date (four NG and four RG) to obtain additional samples for SDS-PAGE. For the carcass halves from the last slaughter date the insoluble pellet following extraction with HIS was also retained for SDS-PAGE.

3.2.6 COOKED BREAST MEAT MEASUREMENTS

3.2.6.1 BATTER FORMULATION AND PREPARATION

The Kjeldahl protein values were used to formulate 200 g batches of meat batter to 14% (wt/wt) protein, 2% (wt/wt) NaCl, and 0.6% (wt/wt) sodium tripolyphosphate (STPP) for torsion testing (Northcutt, 1994). Meat, ice water, salt, and STPP were chopped for 60 s in a food processor (Proctor Silex Canada, Inc., Picton, ON). Temperature following chopping ranged from 3.5 to 5.5°C. Following chopping, the batter was placed in a vacuum bag and a vacuum (-80 kPa) was drawn three times to remove as much air as possible. Preweighed copper tubes (length 15 cm, internal diameter 2.0 cm, tube thickness 1.0 mm, three tubes per sample) were lightly coated inside with a vegetable oil spray (Pam®; International Home Foods Canada Inc., Niagara Falls, ON) and filled with meat batter using a modified caulking gun. The weight of the filled tubes was recorded to determine cook yield. The pH of the raw meat batter was determined as previously described for the ground meat.

Filled tubes were cooked according to the procedure of Boles and Swan (1996). Tubes were tempered for 30 min at ~ -1°C prior to immersion in a 50.0 $\pm$ 0.2°C water bath for 30 min. Tubes were transferred to a 70.0 $\pm$ 0.2°C water bath for 45 min and then chilled in ice water. The tubes were removed from the ice water the following morning (8 to12 h) and allowed to equilibrate to room temperature before removal of the cooked meat batter.

3.2.6.2 COOK YIELD AND EXPRESSIBLE MOISTURE

The cooked gels were removed from the tubes, blotted dry and weighed. Cook yield was expressed as a percentage of the original raw batter weight.

Three discs, ~ 2 mm thick (~1.5 g) were cut from each sample for expressible moisture measurement (Barbut, 1996b). Each disc was placed in a Falcon (Fisher Scientific, Nepean, ON) 50 mL screw cap centrifuge tube fitted with a 212 mesh stainless steel screen (Banjo Corp., Crawfordsville, IN) mounted above the bottom of the tube on a O-ring. Two pieces of filter paper were placed on top of the screen (Whatman™ #50 on top of Whatman™ #3; Whatman Laboratory Division, Maidstone, Kent, UK) with the meat sample placed on top of these. The samples were held at ~ 4°C for at least 1 h and centrifuged at 750 x *g* for 10 min at room temperature. The moisture lost by the sample was determined by the difference in weight of the sample before and after centrifugation, and expressed as a percentage of the weight of the sample before centrifugation.

3.2.6.3 TORSION GELOMETRY

The cooked gels were removed from the copper tubes following equilibration to room temperature, blotted dry, and cut into as many ~ 28.7 mm long cylinders as possible (~12 per sample). Cyanoacrylate glue (Loctite 404; Loctite Corp., Mississauga, ON) was used to attach styrene discs (Gel Consultants Inc., Raleigh, NC) to each end of the cut samples. These samples were milled into capstan-shapes with a center diameter of ~ 10 mm using a modified bench grinder (Gel Consultants

Inc., Raleigh, NC). Following shaping the samples were twisted to fracture at 2.5 rpm on a torsion Gelometer (Gel Consultants Inc., Raleigh, NC) according to methods described by Kim *et al.* (1986). The shear stress and true shear strain at fracture were determined using software which calculates these on the basis of torque and angular displacement (Hamann, 1983).

3.3 ELECTROPHORESIS AND IMMUNOBLOTTING

3.3.1 PREPARATION OF PROTEIN SAMPLES FOR ELECTROPHORESIS

3.3.1.1 SARCOPLASMIC AND MYOFIBRILLAR PROTEINS

The sarcoplasmic and salt soluble protein samples from the postrigor heating study where protein extractability was performed and all 48 carcass halves of the rate of rigor development and delay chilling experiment were prepared for SDS-PAGE. Additionally, the insoluble pellet following extraction with HIS from each half of 8 carcasses (4 RG and 4 NG from last slaughter date of the rate of rigor development experiment) was prepared for electrophoresis. The insoluble pellet was washed 3 times with 5 mM Tris HCl (pH 8.0) and centrifuged at 1,500 x g for 10 min following each wash as Wang (1982) described for washed myofibril preparation. Protein concentration of all samples was determined using the Biuret procedure (Gornall, 1949) with bovine serum albumin as the standard. Samples were adjusted to equal protein concentration and prepared for SDS-PAGE by adding SDS-PAGE sample buffer (8 M urea, 2 M thiourea, 3% (wt/vol) SDS, 0.7 M 2-mercaptoethanol,

M 2-mercaptoethanol, 25 mM Tris-HCl, pH 6.8) as described by Yates and Greaser (1983) in a 1:1 ratio of sample to sample buffer, heated for 20 min at 50 ± 2°C (Boles *et al.*, 1992), and centrifuged for 3 min at 6,600 x *g*.

3.3.1.2 WASHED MYOFIBRIL PREPARATION

Myofibril preparations were used to monitor the degradation of titin, nebulin, and myosin as described by Huff-Lonergan *et al.* (1996). Myofibril samples were prepared from 24 h postmortem breast muscle samples from the last three slaughter dates of the rate of rigor development and delay chilling experiments. The breast muscle samples frozen in liquid nitrogen at 15 min and 3 h postmortem on the last slaughter date were also prepared as washed myofibril samples. Additionally, the postrigor heated samples selected from the breast meat of carcasses selected based on early postmortem pH were used for myofibril preparations. Myofibrils were prepared as described by Goll (1974) and modified by Boles *et al.* (1992). Approximately 300 mg of frozen muscle was shaved into 3 mL of standard salt solution (SSS; 0.1 M KCl, 20 mM potassium phosphate, 2 mM EGTA, 2 mM $MgCl_2$, 1 mM NaN_3, pH 6.7, ~4°C). Protease inhibitors (0.1 mM phenylmethanesulfonyl flouride (Sigma Chemical Company, St. Louis, MO), 2 mg/L leupetin (Calbiochem, San Diego, CA), 1 mg/L pepstatin (Calbiochem), 10 mg/L L-1-tosylamido-2-phenylethyl chloromethyl ketone (ICN Pharmaceuticals Ltd., Montreal, PQ), 10 mg/L trypsin inhibitor (Sigma Chemical Company), 10 mg/L benzylarginylmethyl ester (Sigma Chemical Company)) were added to all buffers

used in the wash procedure. The sample was homogenized for 5 s and centrifuged at 1000 x *g* for 10 min at ~ 2°C. This step was repeated twice, resuspending the pellet first in 2 mL of SSS followed by resuspension in 3 mL of SSS. The pellet was vortexed 5 s in 2 mL of 1% Triton X-100 in SSS, and centrifuged at 1,800 x *g* for 10 min. The Triton X-100 wash of the pellet was repeated. The supernatant was discarded, the pellet was vortexed in 3 mL of SSS and centrifuged at 1,800 x *g* for 10 min. This was repeated using 3 mL of 100 mM KCl instead of SSS. The KCl wash was repeated followed by two washes in 3 mL of 5 mM Tris-HCl (pH 8.0). The final step was to suspend the pellet in 1 mL of 5 mM Tris-HCl (pH 8.0). Protein determination and preparation for SDS-PAGE of the washed myofibrils suspended in Tris-HCl was as described for the sarcoplasmic and myofibrillar protein extraction preparations.

3.3.2 SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS.

SDS-PAGE was performed as outlined by Laemmli (1970) using a Mini-Protein® II electrophoresis unit (Bio-Rad Laboratories, Hercules, CA). Protein samples analyzed included LIS and HIS extracts, residual proteins following extraction by HIS, and the washed myofibril preparations. These samples were loaded at ~ 10 or 20 μg of protein per lane on gels of different acrylamide (Bio-Rad Laboratories) content. Resolving gels of 11% (100:1 acrylamide/N,N'-bis-methylene acrylamide, Bio-Rad Laboratories), 10% (75:1), 8% (100:1), or 7% (100:1) each with a 4% (37.5:1) stacking gel were used. Gels were run at 35

milliamps, constant current until the dye front reached the bottom of the gel. Gels were stained for ~ 30 min in 0.1% Coomassie brilliant blue R-250 (Bio-Rad Laboratories), 10% glacial acetic acid, and 40% methanol. Gels were then destained twice in the same solution without Coomassie blue. Gels used for Western blot analysis were equilibrated in transfer buffer for ~ 15 min prior to transfer. Following transfer of proteins, the polyacrylamide gels were stained with Coomassie blue and destained to determine the efficiency of transfer.

3.3.3 IMMUNOBLOTTING

Following SDS-PAGE the proteins were transferred to nitrocellulose membranes (0.45μm, Bio-Rad Laboratories) at 40 volts for 2.5 h using a Transblot Cell® (Bio-Rad Laboratories). Protein samples included those from the postrigor heating experiments and from the halves of eight NG and eight RG carcasses from last three slaughter dates of the rate of rigor development and delay chilling trial. Samples probed for the larger proteins, titin and nebulin, were transferred at 40 volts for 3 h followed by an additional 2.5 h at 45 volts. The transfer buffer contained 25 mM Tris, 192 mM glycine, 0.1% SDS, and 20% (v/v) methanol (Towbin *et al.*, 1979). Following transfer, the nitrocellulose was washed 10 min in Tris-buffered saline (TBS, 20 mM Tris, 500 mM NaCl, pH 7.5). The nitrocellulose was blocked with 3% gelatin (Bio-Rad Laboratories) in TBS for at least 1 h at room temperature. Following blocking, the membranes were washed twice in TBS containing 0.05% Tween 20 (TTBS) for 5 min each time.

The membranes were then incubated with the primary antibody in 1% gelatin in TBS overnight at room temperature. Membranes were probed for specific proteins using antibodies towards desmin (Clone D78, Iowa State University Hybridoma Bank), creatine kinase (polyclonal towards the MM isozyme of creatine kinase, Biodesign International, Kennebunk, ME), glycogen phosphorylase (clone 10H5, Biodesign International, Kennebunk, ME), glycogen debranching enzyme (clone 9063αDE, gift from Dr. Y. T. Chen, Duke University, Durham, NC), M-Protein (clone A6, gift from Dr. J. C. Perriard, Institute of Cell Biology, Zurich, Switzerland), titin (clone T11, Sigma Chemical Company), nebulin (clone NB1, Sigma Chemical Company), and myosin heavy chain (clone F27 and F59, gifts from Dr. F. E. Stockdale, Stanford University, Palo Alto, CA). Membranes were washed with TTBS twice for 5 min and incubated for at least 1 h at room temperature in 1% gelatin in TBS containing the secondary antibody conjugated to alkaline phosphatase (rabbit anti-goat for the creatine kinase antibodies, goat anti-rabbit for the glycogen debranching enzyme antibody, and goat anti-mouse for the remaining antibodies, Sigma Chemical Company). The membranes were washed twice in TTBS for 5 min followed by a 10 min wash in TBS. The nitrocellulose membranes were agitated on a counter top lab shaker for the duration of blocking and each step involving washing or incubation with antibodies.

Reactivity of the primary antibody with its antigen was detected by incubation of the blots with the alkaline phosphatase substrate. Positive controls were used when probing with the MHC and glycogen phosphorylase antibodies. Development for other antibodies continued until a band of the expected molecular weight

appeared. The substrate solution consisted of 15 mg of 5-bromo-4-chloro-3-indolyl phosphate (Bio-Rad Laboratories) and 30 mg of nitro blue tetrazolium (Bio-Rad Laboratories) were dissolved separately in 1 mL N,N-dimethylformamide and added to 100 mL of 0.1 M $NaHCO_3$, 1.0 mM $MgCl_2$, pH 9.8.

3.3.4 DENSITOMETRY

SDS-PAGE protein bands were scanned using an IS-1000 digital imaging system (Alpha Innotech Corp., San Leandro, CA). Densitometry was performed on the bands in SDS-PAGE gels loaded with HIS samples from eight NG and eight RG carcass halves from the first four slaughter dates.

Samples from carcasses from the last three slaughter dates were probed with all the antibodies in preliminary studies to determine trends. However, densitometry to determine the relative quantity of protein that cross-reacted with the primary antibodies was used on blots containing proteins from the carcasses of the last slaughter date (four NG and four RG). The densitometry results from the Western blot analyses was reported as arbitrary peak area values.

3.4 STATISTICAL ANALYSIS

All means were calculated and analyzed using the Statistical Analysis System (SAS) computer statistical package (SAS Institute Inc., Cary, NC).

3.4.1 POSTRIGOR HEATING PROTEIN EXTRACTABILITY RESULTS

There were five turkey *Pectoralis major* samples used with each turkey considered a block in the analysis. The experimental design was a randomized block design with a 2^5 factorial (two temperatures, two heating times, two extraction buffers, two separation methods, and two days for protein measurements). The error term was the interaction of block with all the independent variables. Analysis was also performed separately on the data from each separation method (filtered samples and centrifuged samples) to determine if there was more error associated with one method. The data from each extraction solution were analyzed separately for similar reasons.

3.4.2 MEAT QUALITY MEASUREMENTS FOR RAPID POSTMORTEM GLYCOLYSIS AND DELAY CHILLING STUDY

Means for each attribute were computed and analyzed as a split plot design using the General Linear Models procedure (SAS Institute Inc., 1990). The rate of rigor development was the main plot treatment with the comparison of chilling immediately vs delayed chilling as a subplot treatment (Table 3.2). Each of the first four slaughter dates were the blocks in the experiment. There were two carcasses from each rate of glycolysis per block. The subsampling terms were tested against the appropriate interactions with block to determine whether these terms were significantly different from each other and whether it was appropriate to combine them for the error terms. The interaction with block and rate of rigor development was combined with the subsampling term (two birds per treatment per block) to

create the error term for testing main plot effects. The interaction with blocks and chilling rate as well as the three way interaction with blocks, rate of rigor development, and chilling rate were combined with the subsampling term to form the error for testing subplot effects. Least squared differences with a P-value of 0.05 were calculated to determine significant differences between treatments. Regression analysis was used to determine correlation coefficients between measurements of colour, pH, protein extractability, expressible moisture, cook yield, and stress and strain at fracture.

Table 3.2 - Analysis of variance table for turkey breast meat quality measurements

Source of Variation	Degrees of Freedom
Block	3
Rate of postmortem glycolysis	1
Carcass half (Block x Rate of postmortem glycolysis)[1]	11
Carcass chilling	1
Rate of postmortem glycolysis x Carcass chilling	1
Carcass half (Block x Rate of postmortem glycolysis x Carcass chilling)[2]	14

[1]Error term for rate of postmortem glycolysis.
[2]Error term for carcass chilling and rate of postmortem glycolysis x carcass chilling.

3.4.3 SDS-PAGE AND WESTERN BLOT DENSITOMETRY RESULTS

Means for the densitometry values of each band in SDS-PAGE gels for the HIS extract from the halves of eight NG and eight RG carcasses (last three slaughter dates) were calculated and analyzed as a split plot design using the General Linear Models procedure (SAS Institute Inc., 1990). The rate of rigor development was the main plot treatment with the comparison of IC versus DC as a subplot treatment. Slaughter dates were the blocks in the experiment.

Means for the densitometry results of Western blots of samples from each of eight carcasses (four RG and four NG; last sampling time) were also analyzed as a split plot design. However, each Western blot performed in duplicate was considered the block in this instance, with a total of four blocks. Each Western blot consisted of samples from four carcass halves, one from each category for the rate of postmortem glycolysis and time of chilling.

4. RESULTS AND DISCUSSION

4.1 PROTEIN EXTRACTABILITY OF POSTRIGOR HEATED TURKEY BREAST MEAT

The results of protein extractability measurements for postrigor turkey breast meat held at 0°C (controls) and 40°C are presented in Figure 4.1. The pH of the breast muscle homogenates prior to heating ranged from 5.40 to 5.70. The extractability of both sarcoplasmic and myofibrillar proteins was reduced following exposure to 40°C. As time at 40°C increased from 30 to 120 min the extractability of each protein fraction decreased even further.

Statistical analysis indicated there was no interaction between the method for measuring protein extractability and heating postrigor turkey breast meat. Therefore, reductions in protein extractability due to postrigor heating were detected when either the filtration method or the centrifugation method was used. However, when the protein extractability measurements determined by filtration were analyzed separately from the centrifugation measurements, it was apparent a larger portion of the error term was associated with the filtration method. The coefficient of variation for extractability measurements in HIS buffer using the filtration method was ~ 2.5x that of the centrifugation method (Table 4.1). The coefficient of variation for sarcoplasmic protein extraction (LIS) was ~ 4x larger for filtration compared to

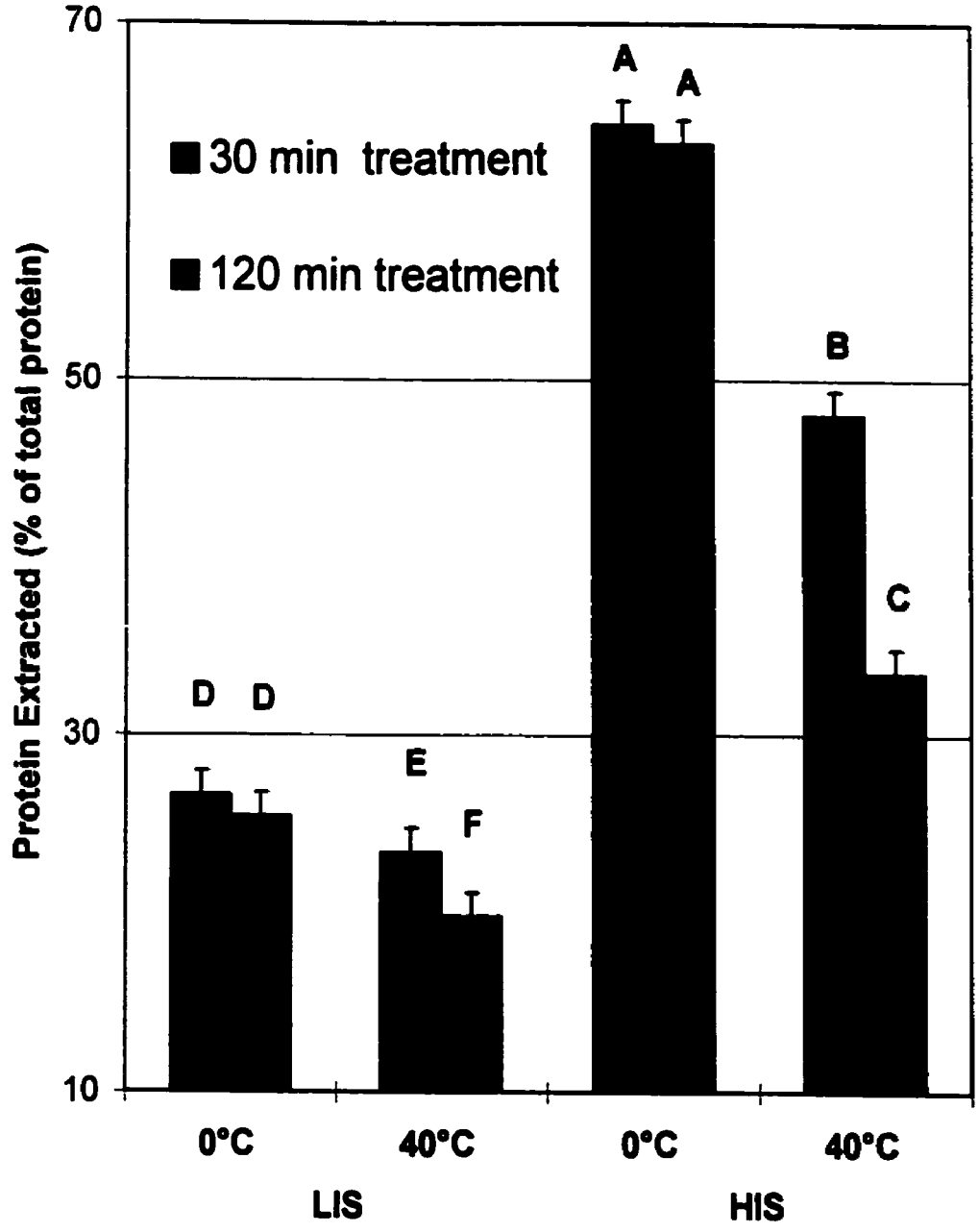

Figure 4.1 - Extractability of proteins in low ionic strength (LIS) and high ionic strength (HIS) buffer from turkey breast meat samples held at 0 or 40°C for 30 or 120 min. Means labeled with different letters are significantly different (P< 0.05). Values are averaged across protein extractability methods and time of measurement.

Table 4.1 - Sarcoplasmic and myofibrillar protein extractability of postrigor turkey breast meat held at 0 and 40°C measured by centrifugation and filtration methods averaged over treatment time

Separation Method	Temperature (°C)	Protein Extracted in LIS[1]	Protein Extracted in HIS[2]
Centrifugation	0	24.1[c]	57.7[a]
Centrifugation	40	20.1[d]	35.5[b]
CV		4.8	6.1
		(n=20)	(n=20)
Filtration	0	27.9[c]	69.8[a]
Filtration	40	23.1[d]	46.1[b]
CV		20.8	14.2
		(n=20)	(n=20)

[1]Percentage of total protein in a sample extracted in low ionic strength buffer (0.05 M potassium phosphate, pH 7.3).
[2]Percentage of total protein in a sample extracted in high ionic strength buffer (0.55 M KCl, 0.05 M potassium phosphate, pH 7.3).
[a-b] Means with different superscripts within a separation method in the same column differ ($P < 0.05$).

centrifugation.

Patel and Fry (1987) reported that the porosity of filters can influence the apparent solubility of proteins. They referred to a study by West *et al.* (1986) in which filtration was compared to centrifugation for separating soluble proteins. West *et al.* (1986) reported significantly higher solubility results for filtration of caseinate and whey protein isolate using Whatman No. 1 filter paper compared to centrifuged samples. In the present study with postrigor heated turkey breast meat, 42% of the total protein in samples was "apparently" extracted using filtration while 34% of the total was extracted with centrifugation. West *et al.* (1986) found no difference between filtration and centrifugation when Whatman No. 5 (slow rate filter) was used. However, in the present study, flow of the turkey breast extraction solutions through Whatman No. 1 filter paper was as slow as 30 to 60 min for some heat treated samples. Using slower filters to improve separation would slow the procedure to an even more unacceptable rate.

Visual observation of protein extraction solutions indicated that the turbidity of filtered samples was greater than the centrifuged samples. Following 48 h of refrigerated storage a precipitate was noticeable in the filtered samples, especially the samples extracted in LIS buffer. Precipitation of proteins resulted in protein extractability measurements that were significantly ($P < 0.05$) lower after 48 h of storage than the initial measurements taken following filtration. A difference between initial and post-storage measurements was observed for proteins extracted with either LIS or HIS buffer when filtered (Figure 4.2). These results suggested

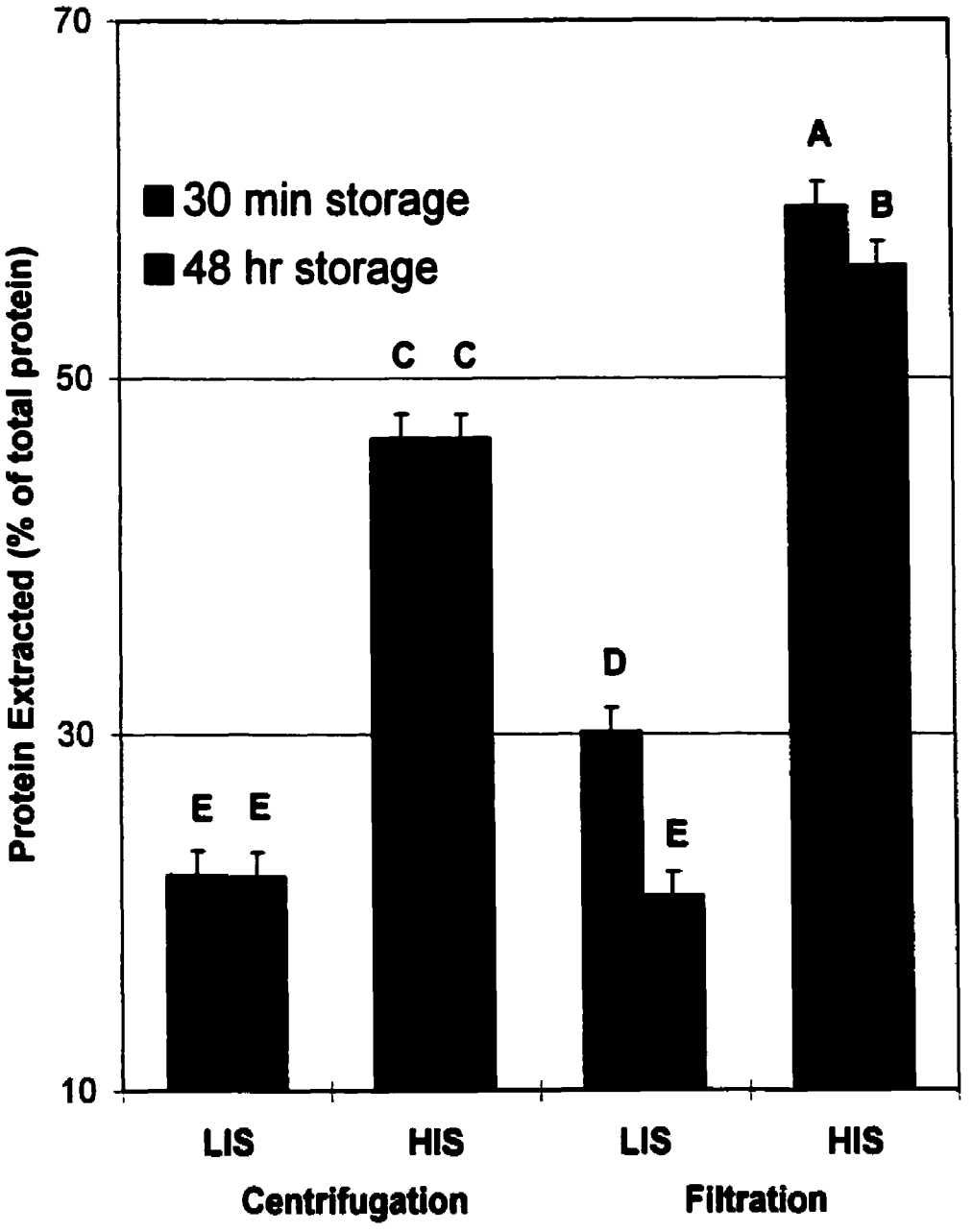

Figure 4.2 - Extractability of proteins in low ionic strength (LIS) and high ionic strength (HIS) buffer following centrifugation at 17,500 x *g* or filtration through Whatman® No. 1 filter paper. Protein extractability was measured 30 min after separation of extractable proteins and 48 h later. Means labeled with different letters are significantly different ($P < 0.05$). Values are averaged across treatment temperatures (0 and 40°C) and time of treatment (30 and 120 min).

that a significant amount of insoluble protein had passed through the filters used in the extraction procedure.

There were no visual signs of precipitation in centrifuged samples and protein concentration measurements made after refrigerated storage were not different from those made 30 min after the extraction procedure. The protein extractability results obtained by centrifugation were considered more reliable than the filtration protein extractability results because of the reduced CV for centrifugation. Therefore, the remainder of the discussion on protein extractability will only include results from centrifuged samples.

For postrigor turkey breast samples held at 40°C for 30 and 120 min, the amount of protein extracted in LIS was reduced by 10 and 23%, respectively, compared to samples held at 0°C (Figure 4.3). These results were similar to the findings of Sakata *et al.* (1983) who reported extractability of sarcoplasmic proteins for postrigor porcine meat held at 40°C for 90 min was reduced 30%. In comparison, Lopez-Bote *et al.* (1989) reported that sarcoplasmic protein extracted from PSE pork was reduced 16% compared to normal meat.

Fischer *et al.* (1979) and Sakata *et al.* (1981,1983) held postrigor porcine muscle at 40°C as a model to produce PSE-like meat. Although they measured sarcoplasmic protein extractability, they did not investigate the effect of this treatment on the extraction of salt-soluble proteins. The proteins extracted in HIS buffer are more important to product quality than are sarcoplasmic proteins (Pearson and Young, 1989). In the present study, when postrigor turkey breast muscle samples were placed in 40°C water for 30 min, protein extracted with HIS was 70%

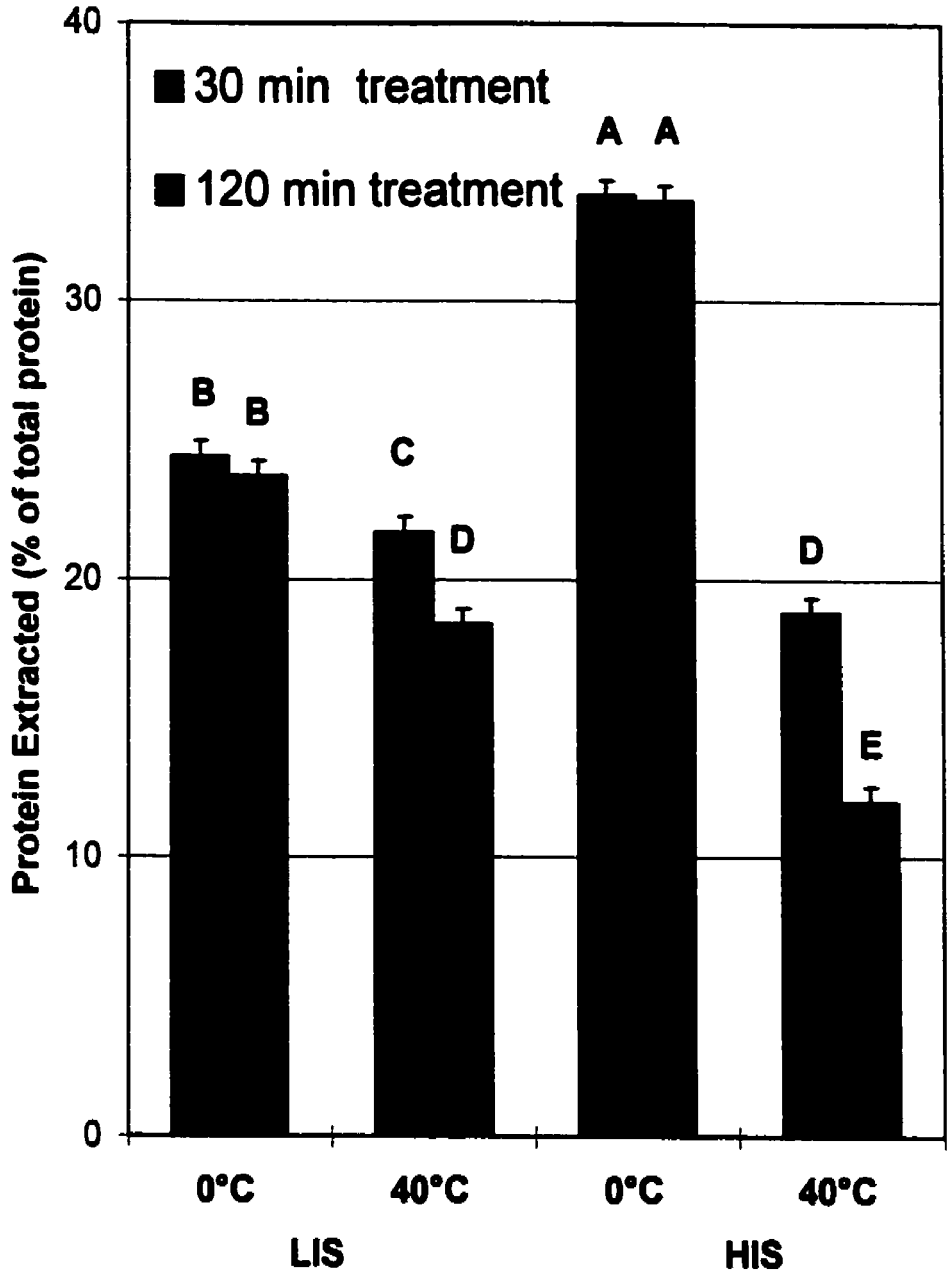

Figure 4.3 - Extractability of proteins in low ionic strength (LIS) and high ionic strength (HIS) buffer from turkey breast meat samples held at 0 or 40°C for 30 or 120 min. Means labeled with different letters are significantly different ($P<0.05$). Values are from centrifugation results averaged across time of measurement.

of the amount extracted at 0°C. When held at 40°C for 120 min, protein extracted with HIS was reduced to 53% of controls (Figure 4.3). Lopez-Bote *et al.* (1989) reported that myofibrillar protein extractability for PSE porcine meat was 59% of that extracted from normal meat. Lopez-Bote *et al.* (1989) determined myofibrillar protein extractability by subtracting the amount of protein extracted in low ionic strength buffer from the total protein extracted with high ionic strength buffer. Using this calculation for the results in the current study, the myofibrillar protein extractability was reduced to 54% and 36% of the controls after 30 and 120 min at 40°C respectively. These results indicate that the extractability of myofibrillar proteins in postrigor turkey breast meat was more sensitive to conditions of low pH and elevated temperatures than the extractability of sarcoplasmic proteins.

Regardless of the method used to measure protein extractability, holding postrigor turkey breast meat at 40°C for 30 to 120 min reduced the extractability of both sarcoplasmic and myofibrillar proteins. Holding postrigor turkey breast muscle at 40°C reduced extractability of the myofibrillar proteins to a greater extent than sarcoplasmic proteins. This reduction in protein extractability in breast muscle samples held at 40°C was similar to the reduction in protein extractability reported for porcine muscle and turkey breast muscle with abnormally rapid postmortem rigor development (Lopez-Bote *et al.*, 1989; Boles *et al.*, 1992; van Laack *et al.*, 1993; Pietrzak *et al.*, 1997).

4.2 QUALITY OF RAPID GLYCOLYZING AND DELAY CHILLED TURKEY BREAST

The following study focused on the influence of the rate of pH decline in turkey breast meat and a delay in chilling of carcasses on meat quality measurements. The measurements of breast meat quality included colour, protein extractability, cook yield, expressible moisture, and gel strength. Statistical analysis of the results of these measurements indicated there were no interactions between rate of pH decline and carcass chilling rate. Therefore, only differences between main effects will be discussed.

4.2.1 CARCASS TEMPERATURE

Carcasses selected as RG based on low 15 min postmortem pH values were found to have a significantly higher ($P < 0.05$) carcass temperature at 15 min postmortem than NG carcasses (Table 4.2). Studies involving pork have made similar findings in which early postmortem temperature for PSE pork was higher than for normal pork due to heat generated by accelerated metabolism (Offer, 1991). The difference in temperature decline between carcass halves immediately immersion chilled and halves delayed chilled are readily apparent in Figure 4.4. The average temperature at the center of the breast muscle following the 90 min delay in chilling was 39.8 $\pm$ 1.2°C. Following 90 min in the insulated box, the rate of temperature decline for the delay chilled carcass halves (0.31 $\pm$ 0.06°C/min, n=3) was similar to halves which were immediately chilled (0.33 $\pm$ 0.06°C/min, n=3). The average

TABLE 4.2 - Carcass temperature, ultimate pH and colour measurements of normal and rapid glycolyzing turkey breast and the effect of delaying carcass chilling until 110 min postmortem

Source	Carcass temp 15 min postmortem (°C)	Ultimate pH	L^*	a^*	b^*
NG[1]	43.9[b]	5.71[a]	52.01[a]	15.66[a]	14.02[a]
RG[2]	44.5[a]	5.67[a]	51.86[a]	17.04[a]	14.90[a]
SEM (n=8)	0.15	0.02	0.54	0.46	0.42
IC[3]		5.73[a]	51.06[b]	15.80[b]	13.68[b]
DC[4]		5.65[b]	52.81[a]	16.90[a]	15.23[a]
SEM (n=16)		0.01	0.19	0.20	0.23

[1] NG = Normal glycolyzing (pH > 6.00 at 15 min postmortem).
[2] RG = Rapid glycolyzing (pH ≤ 5.80 at 15 min postmortem).
[3] IC = Immediately chilled (20 min postmortem).
[4] DC = Delay chilled (110 min postmortem).
[a-b] Means in a column followed by the same superscript within a treatment group are not significantly different ($P < 0.05$).

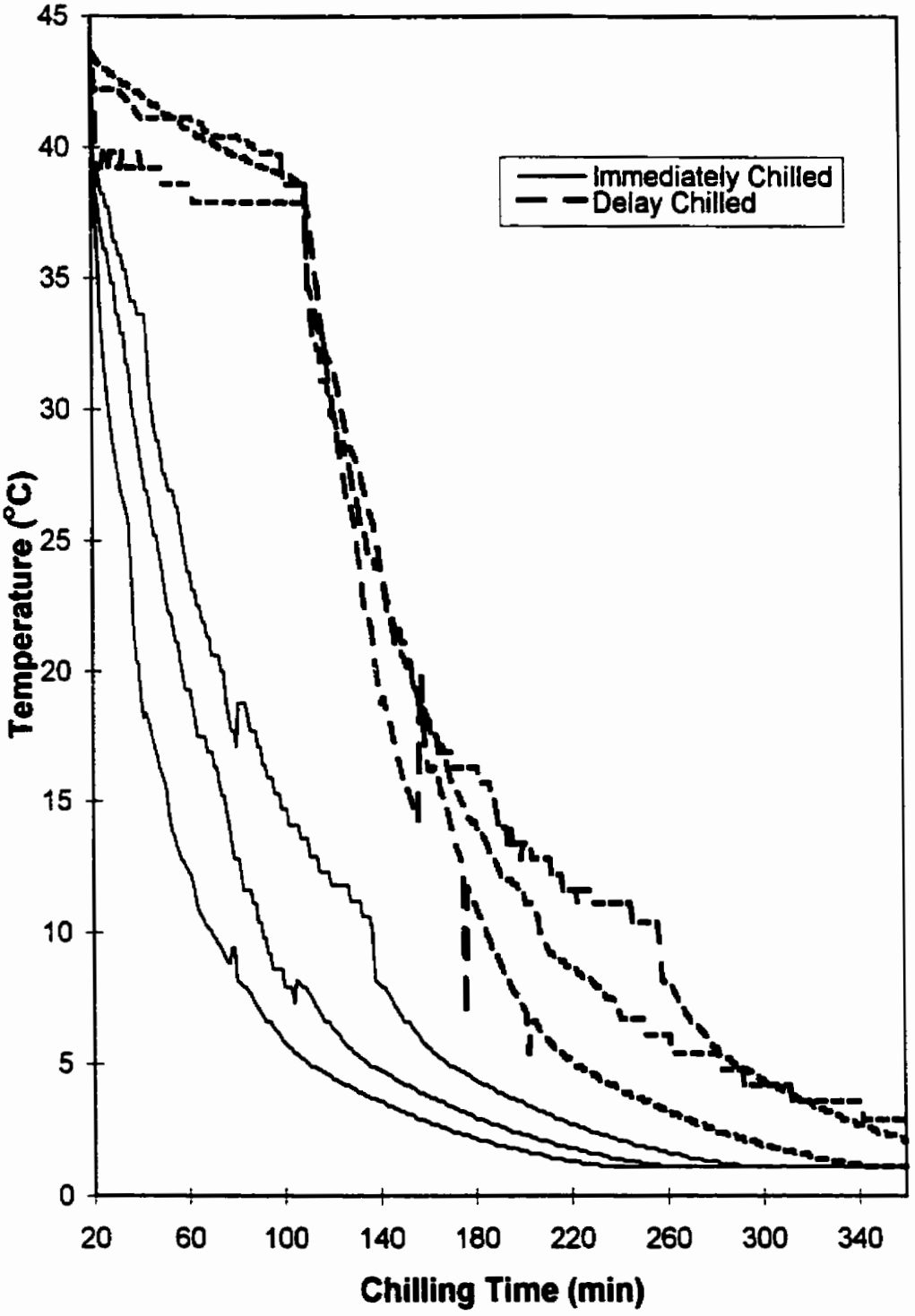

Figure 4.4 - The temperature of turkey breast muscle from three slaughter dates (n=3) chilled at 20 min postmortem or delayed until 110 min postmortem.

breast temperatures for delayed and immediately chilled carcass halves after 60 min of chilling were 20.4 ± 2.7°C and 21.5 ± 3.2°C, respectively.

4.2.2 MUSCLE pH AND COLOUR

Comparison of 15 min pH values with ultimate pH indicated that for some RG carcasses rigor was complete at 15 min postmortem as no further decline in breast muscle pH was observed. The ultimate pH values of the breast meat from the RG carcasses were not different from those of the NG carcasses (Table 4.2; 4.3). One of the goals of this study was to evaluate the effects of the rate of pH decline without the influence of differences in ultimate pH. This was achieved by excluding carcasses with extreme ultimate pH values from each block. However, delaying the chilling procedure for one half of the carcass did reduce the ultimate pH (pH difference = 0.08) compared to halves chilled immediately (Table 4.2).

Colour of ground breast meat was not affected by rate of postmortem glycolysis (Table 4.2). However, there was a trend ($P = 0.055$) for breast meat from RG carcasses to have higher a^* values than NG samples. Several researchers have reported colour changes in turkey breast meat that underwent an accelerated rate of postmortem glycolysis (Froning *et al.*, 1978; McKee and Sams, 1997; Pietrzak *et al.*, 1997). Froning *et al.* (1978) reported lower L values and higher a values for breast meat from heat-stressed or free struggling turkeys compared to the control birds. Pietrzak *et al.* (1997) found higher L^* values for turkey breast meat designated PSE based on 20 min postmortem pH values of less than 5.8 compared to normal meat

Table 4.3 - The average breast muscle pH at 15 min postmortem for normal and rapid glycolyzing turkey carcasses and the breast muscle pH for carcasses selected 36 h postmortem

Day	Carcasses selected 15 min postmortem	pH 15 min postmortem	Carcasses selected 36 h postmortem	pH 36 h postmortem
1	4 NG[1]	6.08 ± 0.05[3]	2 NG	5.66 ± 0.08[3]
	2 RG[2]	5.74 ± 0.03	2 RG	5.66 ± 0.10
2	3 NG	6.13 ± 0.08	2 NG	5.83 ± 0.04
	2 RG	5.80 ± 0.05	2 RG	5.68 ± 0.14
3	2 NG	6.10 ± 0.05	2 NG	5.69 ± 0.08
	3 RG	5.63 ± 0.03	2 RG	5.66 ± 0.04
4	2 NG	6.10 ± 0.04	2 NG	5.66 ± 0.08
	4 RG	5.76 ± 0.03	2 RG	5.64 ± 0.09
5	4 NG	6.09 ± 0.10	4 NG	5.71 ± 0.06
	4 RG	5.73 ± 0.08	4 RG	5.70 ± 0.06
Total	15 NG	6.10 ± 0.07	12 NG	5.71 ± 0.06
	15 RG	5.73 ± 0.05	12 RG	5.67 ± 0.08

[1]NG = Normal glycolyzing.
[2]RG = Rapid glycolyzing.
[3] ± = Standard deviation.

(pH > 5.8), but no differences in a^* values were found. McKee and Sams (1997) reported increased L^* values for breast meat from turkey toms exposed to high temperatures (38/32°C - day/night) for the last 4 wk of life compared to control birds kept at cooler temperatures.

It is difficult to determine whether increases in L^* value in the studies by Pietrzak *et al.* (1997) and McKee and Sams (1997) are due to accelerated postmortem glycolysis or to differences in ultimate pH or both. The ultimate pH was not determined in the study by Pietrzak *et al.* (1997). The ultimate pH for breast meat from heat-stressed birds in the study by McKee and Sams (1997) was 0.10 units lower than for unstressed birds. A significant negative correlation between L^* values and ultimate pH has been reported for turkey breast (Barbut 1993, 1996a) and meat from other animals such as cattle (MacDougall and Rhodes,1972). It must be remembered that colour measurements reported by other investigators were performed on intact muscle tissue rather than ground muscle as was the case in the current study.

Breast meat from delay chilled carcasses was significantly lighter (higher L^*), redder (higher a^*), and more yellow (higher b^*) than comparable samples from carcass halves chilled immediately (Table 4.2). McKee and Sams (1998a) reported increased L^* values for turkey breast held at 40°C for 2 h compared to samples held at lower temperatures (20 and 0°C).

Reasons for these changes in colour with delays in chilling are unclear. The difference in ultimate pH of 0.08 units may contribute to lighter meat. In a study by Cornforth and Egbert (1985) all three colour values increased when prerigor bovine

muscle was acidified with 0.2 M citrate buffer, pH 5.3, or when rotenone was added. Rotenone inhibits mitochondrial activity, allowing myoglobin to remain oxygenated and treated meat to appear a brighter red (Cornforth and Egbert, 1985). Mitochondria from PSE pork show considerable disruption and have an open-structured appearance (Dutson *et al.*, 1974). If mitochondrial activity in turkey breast meat from carcasses proceeding through rigor at an accelerated rate was reduced, there would be less competition with myoglobin for oxygen after grinding and the meat would appear redder.

4.2.3 PROTEIN EXTRACTABILITY

Extractability of both sarcoplasmic (SP) and myofibrillar proteins (MP) from breast meat of RG turkey carcasses was reduced compared to NG carcasses (Table 4.4). While the sarcoplasmic protein extractability was slightly lower in RG carcasses than NG carcasses, the difference in MP extractability between NG and RG carcasses was much larger. The MP extractability of breast meat from NG carcasses was 50% more than RG carcasses. Lopez-Bote *et al.* (1989) found MP extractability was reduced to a greater extent than SP extractability in PSE pork. Boles *et al.* (1992) found similar results for stress-susceptible swine. Northcutt (1994) selected pale and normal coloured turkey breast with the same ultimate pH and found no difference in total (SP+MP) protein extractability.

Delayed chilling of carcasses reduced SP and MP extractability to the same degree as increased rate of rigor development (Table 4.4). Fernandez *et al.* (1994)

TABLE 4.4 - Protein extractability, water holding capacity and rheology of cooked gels from normal and rapid glycolyzing turkey breast and immediately and delay chilled carcasses

Source	Extractable sarcoplasmic proteins[5] (%)	Extractable myofibrillar proteins[5] (%)	Cook yield[6] (%)	Expressible moisture[7] (%)	Stress (kPa)	True strain
NG[1]	29.9[a]	14.6[a]	90.6[a]	20.3[a]	37.47[a]	1.43[a]
RG[2]	27.7[b]	9.7[b]	88.2[a]	20.5[a]	34.83[a]	1.27[b]
SEM (n=8)	0.59	1.11	0.91	0.88	1.80	0.04
IC[3]	29.5[a]	14.4[a]	91.0[a]	20.1[a]	37.99[a]	1.45[a]
DC[4]	28.1[b]	9.9[b]	87.9[b]	20.8[a]	34.30[b]	1.25[b]
SEM (n=16)	0.19	0.62	0.54	0.75	1.28	0.02

[1] NG = Normal Glycolyzing (pH > 6.00 at 15 min postmortem).
[2] RG = Rapid Glycolyzing (pH ≤ 5.80 at 15 min postmortem).
[3] IC = Immediately chilled (20 min postmortem).
[4] DC = Delay chilled (110 min postmortem).
[5] Expressed as a percentage of total protein in the meat sample.
[6] Expressed as a percentage of raw batter weight.
[7] Expressed as a percentage of cooked gel weight.
[a-b] Means with different superscripts within a source and in the same column differ ($P < 0.05$).

subjected pork to different rates of chilling and found a reduction in water and salt soluble protein extraction when the chilling procedure was slower. As ultimate pH increased, the effect of slower chilling rates on protein extractability diminished.

Lesiak *et al.* (1996) removed breast muscle strips immediately postmortem from normal glycolyzing turkey toms and held them at 30°C for 15 and 240 min. They found holding muscle strips at 30°C increased the protein extractability compared to strips held at 0°C. Differences in sample preparation (muscle strip vs intact muscle), treatment temperatures, and protein extractability procedures may all contribute to these contrasting findings.

4.2.4 COOK YIELD AND EXPRESSIBLE MOISTURE

Rate of glycolysis had no effect on cook yield or expressible moisture of cooked meat gels (Table 4.4). This is in contrast to the results of the study by Pietrzak *et al.* (1997) who reported a cook yield of 107.1% for ground RG turkey compared to 126.1% for NG turkey breast. However, the method Pietrzak *et al.* (1997) used to measure cook yield was much different than the one used in this study. They added ground breast meat to a solution of 2.5% NaCl and 0.04 M Na_2HPO_4, pH 6.5 and then centrifuged for 15 min at 1,900 x *g*. The pellet was heated to 80°C and cook yield determined. In addition to differences in methods for determining cook yield the ultimate pH values of the breast meat were not reported in the study by Pietrzak *et al.* (1997). McCurdy *et al.* (1996) reported a significant

negative correlation between ultimate pH of minced turkey breast meat and cook loss.

Delaying the chilling procedure reduced cook yield but had no effect on expressible moisture. A 3% change in cook yield is small but considering the large volume of turkey breast meat processed, this represents an important economic loss. In the study by Lesiak *et al.* (1996), cook yield was reduced for turkey breast muscle strips held at 30°C compared to 0 and 12°C when water, salt, and phosphate were added to the sample. However, no differences were found for samples when only water was added.

4.2.5 STRESS AND STRAIN AT FRACTURE USING TORSION GELOMETRY

Torsion results indicate that the structure of cooked meat gels was affected by differences in both rate of postmortem glycolysis and rate of chilling (Table 4.4). Stress at fracture is a measure of gel hardness and is sensitive to changes in protein functionality, protein concentration, and thermal processing (Hamann, 1988). Stress values can be used to detect when fillers such as starch or denatured proteins are present in a gel prior to cooking. Strain is an indication of deformability or cohesiveness of proteins in the gel (Hamann, 1988). The stress at fracture was not different between gels from RG and NG carcasses. The strain values for RG breast meat gels were lower than gels made from NG meat (Table 4.4). This would suggest the proteins in RG breast meat formed a less cohesive gel than gels from NG birds.

No reports of differences in the stress or strain at fracture between NG and RG turkey breast meat gels are available. However, Northcutt (1994) found no difference in stress and strain values between gels made from pale or normal coloured turkey breast muscles with similar ultimate pH.

Delaying the initiation of chilling reduced both stress and strain of turkey breast meat gels. The lower value for MP extractability would indicate less protein would be available to form gels, resulting in a weaker gel. Changes to stress and strain would explain the poor texture reported for products made from pale soft turkey breast (Ferket, 1995). Camou and Sebranek (1991) produced gels from the salt soluble protein extracts of normal and PSE pork. The gels were back extruded with a plunger attached to an Instron testing device. They reported the strength of gels from PSE meat was 45% of that for gels from normal pork for the same protein concentration.

4.2.6 TREATMENT COMBINATION

No interactions between the rate of rigor mortis development and initiation of the chilling procedure were detected for any measurements made. The practical significance of this is that a delay in carcass chilling produced deleterious effects on carcass quality regardless of how fast the pH was declining. Both rate of rigor and rate of temperature decline affected breast meat quality, a lack of interaction indicates the effects of rapid glycolysis and delayed chilling are additive. Therefore, a delay in chilling would magnify problems already present due to a rapid rate of pH

decline. For example, the MP extractability of immediately chilled NG carcass halves (16.9%) was more than twice that of RG carcass halves delay chilled (7.4%). It is difficult to separate these two processes, as a reduced rate of chilling increases the rate of postmortem glycolysis (Greaser, 1986).

4.2.7 CORRELATION BETWEEN MEAT QUALITY MEASUREMENTS

Correlation coefficients among pH, colour and meat quality measurements are listed in Table 4.5. Correlation coefficients were calculated to evaluate the use of L^* values for predicting changes to meat quality. There were some statistically significant correlations between L^* values and other measurements; however, the r^2 values are less than 0.4 indicating the statistical model does not explain a great deal of the variation. Barbut (1993,1996a) reported significant correlations between L^* value and ultimate pH (r= -0.71 and -0.65 respectively) in investigations where lightness values were used to screen for "PSE turkey breast". He found that higher L^* values also corresponded to breast meat with lower water holding capacity. The results of the current study indicate that the correlation between L^* values and ultimate pH was not very meaningful, with a r-value of only -0.41 ($P < 0.01$). These low correlation coefficients are likely due in part to the small range of ultimate pH values because carcasses with similar ultimate pH values were compared. The lower water holding capacity values in Barbut's (1993,1996a) investigations may have been due to differences in ultimate pH rather than anything related to the rate of postmortem pH decline, as the rate of glycolysis was not determined.

TABLE 4.5 - Correlations (r-values) of measurements from normal and rapid glycolyzing turkey breast including carcass halves chilled immediately and delayed 90 min from chilling (n = 32)

	Ultimate pH	Sarcoplasmic[1]	Myofibrillar[2]	Cook yield[3]	Expressible moisture[4]	Stress	Strain
*L**	-0.41**	-0.47**	-0.59***	-0.37*	NS	-0.45**	-0.61***
*a**	NS	-0.68***	-0.55***	NS	NS	NS	-0.41**
*b**	-0.47**	-0.66***	-0.64***	-0.39*	NS	NS	-0.56***
Sarcoplasmic[1]	0.54***		-	-	-	-	-
Myofibrillar[2]	0.67***	0.71***		-	-	-	-
Cook yield	0.63***	0.53**	0.78***		-	-	-
Expressible moisture[3]	NS	NS	NS	NS		-	-
Stress	NS	NS	NS	NS	-0.72***		-
Strain	0.69***	0.60***	0.81***	0.84***	NS	0.58***	

[1] Extractable sarcoplasmic proteins expressed as a percentage of total protein.
[2] Extractable myofibrillar proteins expressed as a percentage of total protein.
[3] Cook yield expressed as a percentage of the raw batter weight.
[4] Expressible moisture expressed as a percentage of cooked gel weight.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
NS = Not Significant.

Correlation coefficients were also calculated to determine the influence of ultimate pH values on other meat quality measurements. Significant correlations with ultimate pH besides L^* values included, b^* values, SP and MP extractability, cook yield, and true strain. However, most of these correlations were also very low.

Some more meaningful significant correlations resulted between MP extractability, cook yield, and true strain. These positive correlations indicated that as MP extractability increased, cook yield, and strain of breast meat gels also increased. These results indicated that for carcasses selected with similar ultimate pH values MP extractability was a better predictor of gel cohesiveness than the colour measurements used in this study.

4.3 ELECTROPHORESIS AND IMMUNOBLOTTING

4.3.1 RAPID GLYCOLYZING AND DELAY CHILLED TURKEY BREAST

The following study focused on differences in the extractability and degradation of individual muscle proteins from turkey breast meat exhibiting different rates of postmortem glycolysis and from carcasses chilled at different times postmortem. Statistical analysis of the densitometry results of both SDS-polyacrylamide gels and Western blots was used to evaluate results.

4.3.1.1 ELECTROPHORETIC BANDING PATTERNS

The turkey breast muscle proteins extracted in LIS and HIS buffers and the proteins insoluble after HIS extraction were analyzed by SDS-PAGE. There was no

detectable difference in the banding pattern for proteins extracted in the LIS buffer for 8% (100:1) gels (Figure 4.5) or for gels of acrylamide content up to 14% (which would include proteins as small as 15 kDa) regardless of the rate of postmortem glycolysis or time of chilling. However, there were obvious differences in banding patterns due to the rate of postmortem glycolysis and time of chilling for both proteins extracted in HIS buffer and those in pellet samples (Figure 4.5). Of particular interest were the proteins with molecular weights of 95, 133, 142, and 165 kDa. The 133 and 142 kDa bands were never observed for LIS samples but were present in gels containing the HIS and insoluble pellet samples from several turkey carcasses. Protein bands at 133 and 142 kDa were observed in the extractable myofibrillar fraction for 7 of 12 RG/IC carcass halves, 6 of 12 NG/DC carcass halves, and 11 of 12 RG/DC carcass halves. These bands were not detected in the HIS extracts from NG/IC carcass halves.

The intensity of two bands corresponding to proteins of 95 and 165 kDa in each extract was different depending on the classification of the carcass half. For the insoluble pellet, the 95 and 165 kDa bands were more intense in the RG and DC samples compared to NG/IC samples. The presence of these bands in the LIS extract suggested that these proteins may be sarcoplasmic proteins which were not completely extracted in LIS buffer when postmortem glycolysis was rapid or when chilling was delayed. Another possibility is that these proteins are fragments of larger myofibrillar proteins with the same molecular weight as proteins in the LIS extract.

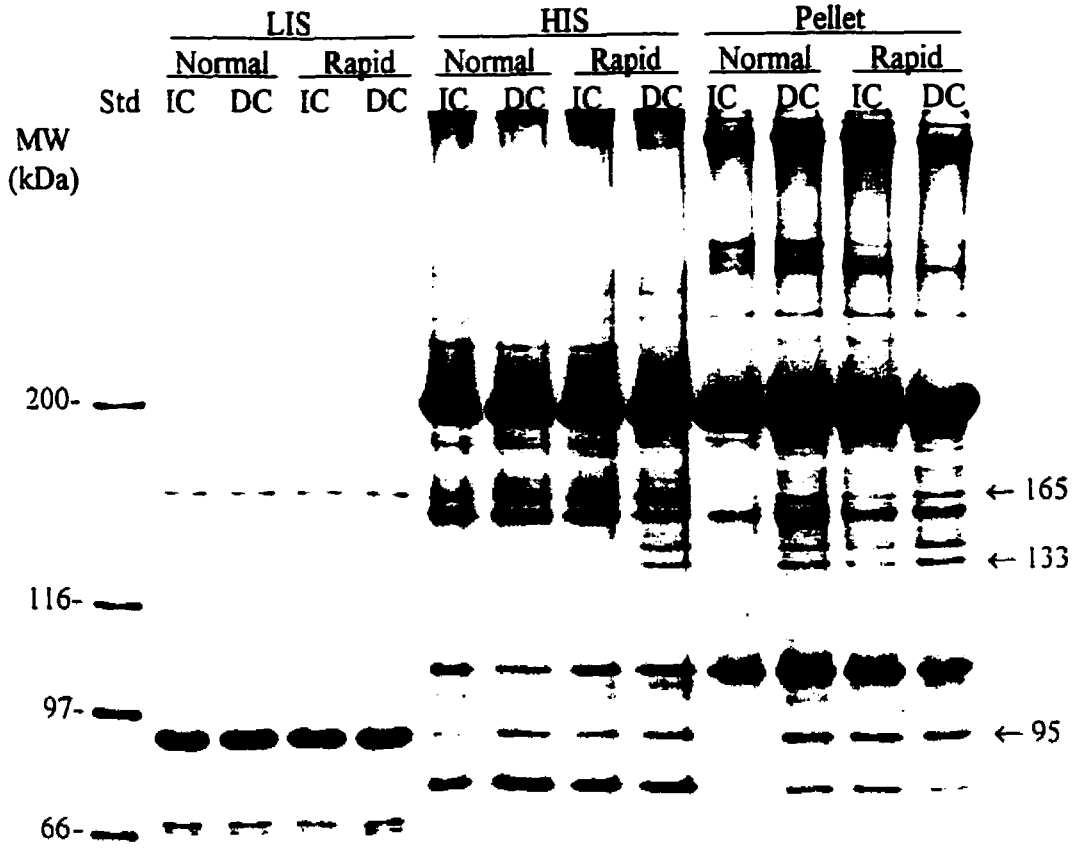

Figure 4.5 - SDS-PAGE patterns of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC) on an 8% (100:1) gel stained with Coomassie brilliant blue. Each lane contains 20 μg of protein from extraction with low ionic strength (LIS) buffer, high ionic strength (HIS) buffer, or proteins insoluble in HIS (Pellet).

4.3.1.2 WESTERN BLOTTING

To identify the proteins (95, 133, 142, and 165 kDa) responsible for the observed differences in SDS-PAGE banding patterns, Western blotting was performed using antibodies of proteins with molecular weights of approximately 95 kDa (glycogen phosphorylase) and 165 kDa (M-protein and glycogen debranching enzyme). In the event that bands were degradation products of larger proteins, samples were probed with antibodies to titin, nebulin, and skeletal muscle myosin heavy chain. Use of several antibodies revealed potential differences in the extractability and/or degradation of specific proteins. Statistical analysis of densitometry results for Western blots was used to objectively evaluate relative differences in protein quantity between treatments. Although this technique is widely used for this purpose, the semiquantitative nature of Western blotting does not allow determination of absolute protein quantity because band saturation can result in a nonlinear relationship between protein quantity and band intensity (Towbin *et al.*, 1979; Jones *et al.*, 1995; Werner and Nagel, 1997). In this study, relative differences may have been underestimated due to potential saturation of some bands.

4.3.1.2.1 GLYCOGEN PHOSPHORYLASE AND CREATINE KINASE

The antibody to the sarcoplasmic protein glycogen phosphorylase reacted with the 95 kDa protein in the LIS and HIS extracts and insoluble pellet samples (Figure 4.6). Observation of Western blot results suggested that there were

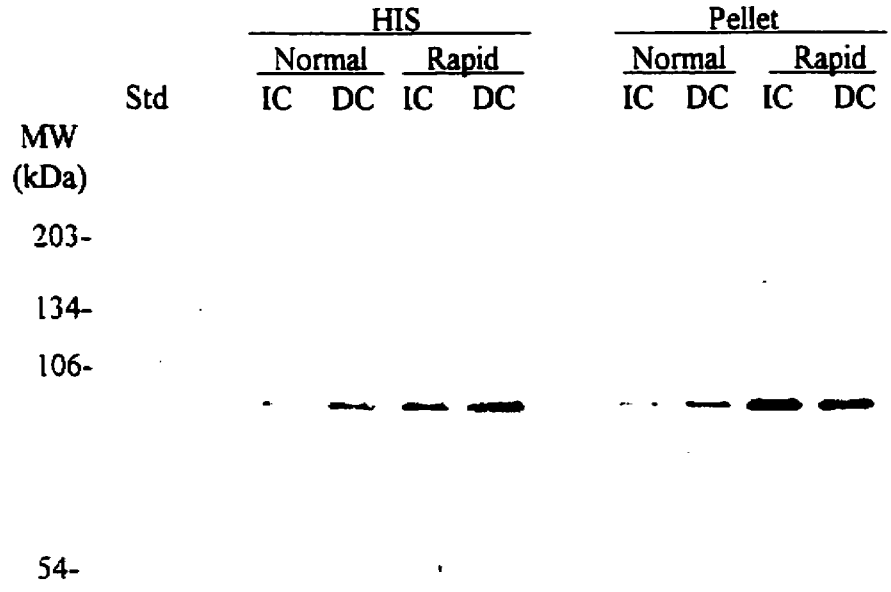

Figure 4.6 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 11% (100:1) gel, and probed with anti-glycogen phosphorylase. Each lane contains 20 μg of protein from extraction with high ionic strength (HIS) buffer or proteins insoluble in HIS (Pellet).

differences in the amount of glycogen phosphorylase in HIS extracts and pellet samples. Statistical analysis of densitometry results indicated a significant increase ($P < 0.05$) in glycogen phosphorylase in the HIS extract of both RG and DC carcasses compared to NG and IC carcass halves (Table 4.6; Table 4.7). The insoluble pellet samples of RG carcasses also had a significant increase of glycogen phosphorylase compared to NG carcasses. There was no significant interaction between the rate of glycolysis and the time of carcass chilling, suggesting effects were additive.

Heizmann and Eppenberger (1978) used immunofluorescence to demonstrate that glycogen phosphorylase was bound to myofibrils in the I-band region and the H-zone in chicken breast muscle. Washing skeletal muscle with low ionic strength buffer is normally sufficient to remove these enzymes from the myofibril (Pearson and Young, 1989). However, several investigators have reported that glycogen phosphorylase was not fully extracted by low ionic strength buffers in PSE pork (Offer, 1991) and RG turkey breast (Pietrzak *et al.*, 1997).

It has been suggested that another sarcoplasmic protein, creatine kinase, also is not completely extracted by low ionic strength buffer in PSE pork (Offer, 1991) and RG turkey breast (Pietrzak *et al.*, 1997). Creatine kinase is a 42 kDa protein with dual roles, functioning as an enzyme and as a structural protein of the M-line in myofibrils (Grove *et al.*, 1985). Significantly greater amounts of creatine kinase were observed in HIS extract of RG carcasses than in samples from NG controls. Results for densitometry of Western blots probed with anti-creatine kinase antibodies are listed in Table 4.6 and Table 4.7.

Table 4.6 - Arbitrary peak area values from densitometry of Western blots for proteins from normal and rapid glycolyzing turkey breast

Rate of Postmortem Glycolysis	Protein Sample	Glycogen Phosphorylase	Creatine Kinase	M-protein
NG[1]	HIS[3]	77.3	90.6	94.9
RG[2]	HIS	122.6	110.1	105.9
P-value		0.003	0.002	0.36
SEM (n=4)		6.88	2.8	14.5
NG[2]	Pellet[4]	105.8	8.5	30.5
RG[2]	Pellet	197.0	12.0	38.1
P-value		0.02	0.14	0.07
SEM (n=4)		30.5	2.5	3.8

[1] NG = Normal Glycolyzing (pH > 6.00 at 15 min postmortem).
[2] RG = Rapid Glycolyzing (pH ≤ 5.80 at 15 min postmortem).
[3] HIS = High ionic strength extract (0.55M KCl).
[4] Pellet = Proteins insoluble in HIS.

Table 4.7 - Arbitrary peak area values from densitometry of Western blots for proteins from turkey breast immediately chilled or delaying chilled

Start of Immersion Chilling	Protein Sample	Glycogen Phosphorylase	Creatine Kinase	M-protein
IC[1]	HIS[3]	91.3	94.8	102.5
DC[2]	HIS	108.6	106.0	98.3
P-value		0.04	0.29	0.69
SEM (n=8)		6.7	9.7	10.2
IC[1]	Pellet[4]	124.8	10.4	31.0[a]
DC[2]	Pellet	178.0	10.1	37.6[b]
P-value		0.14	0.91	0.01
SEM (n=8)		31.3	2.1	1.6

[1] IC = Immediately chilled (20 min postmortem).
[2] DC = Delay chilled (110 min postmortem).
[3] HIS = High ionic strength extract (0.55M KCl).
[4] Pellet = Proteins insoluble in HIS.

The increase of glycogen phosphorylase and creatine kinase in HIS extracts from RG and DC turkey breasts may be the result of increased binding of these proteins to the myofibrils and/or a reduction in extractability due to denaturation. Both glycogen phosphorylase and creatine kinase have been suspected of binding to myofibrils and reducing the extractability of the myofibrillar proteins of PSE pork (Offer, 1991). The Western blot results of the current study indicate an increased association of these two sarcoplasmic proteins with turkey myofibrillar proteins, but conclusions regarding the association of these proteins to reduced myofibrillar protein extractability cannot be made from these results.

4.3.1.2.2 GLYCOGEN DEBRANCHING ENZYME

The glycogen debranching enzyme is a sarcoplasmic protein with a molecular weight of ~174 kDa (Wu *et al.*, 1996). The antibody to glycogen debranching enzyme reacted with the 165 kDa band in LIS extracts but did not react with any bands in HIS extracts or insoluble pellet samples (Figure 4.7). This indicates that extraction of glycogen debranching enzyme in LIS was not affected by the rate of postmortem glycolysis or time of chilling. The apparent reaction of the glycogen debranching antibody with a band of 200 kDa in the HIS extracted and insoluble pellet samples observed in Figure 4.7 was due to over-development of the Western blot.

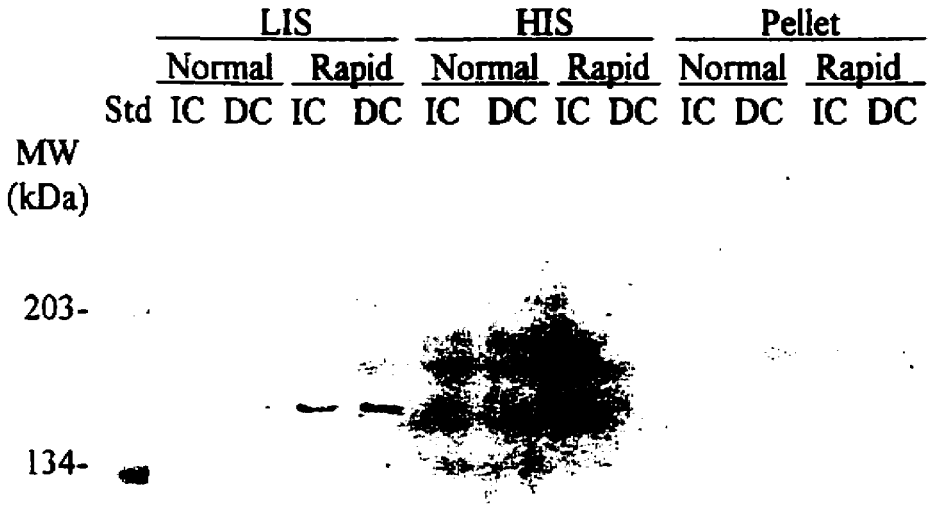

Figure 4.7 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 8% (100:1) gel, and probed with anti-glycogen debranching enzyme. Each lane contains 20 μg of protein from extraction with low ionic strength (LIS) buffer, high ionic strength (HIS) buffer, or proteins insoluble in HIS (Pellet).

4.3.1.2.3 M-PROTEIN

M-Protein is a structural myofibrillar protein of ~165 kDa (Grove *et al.*, 1985) that together with creatine kinase and myomesin constitute the M-filaments of the myofibril (Pearson and Young, 1989), which provide a scaffold to align the thick filaments at the M-line (Grove *et al.*, 1985). Therefore, changes to the extractability of M-line proteins may affect the stability of the myofibril, thereby influencing the extractability of other myofibrillar proteins.

When samples were probed with anti-M-protein antibodies, reactivity occurred with a band ~170 kDa in size. Figure 4.8 shows a Western blot of HIS-extracted proteins and proteins in the insoluble pellet probed with anti-M-protein. Although the 165 kDa band was not identified as M-protein, a significant increase of M-protein ($P < 0.01$) in the insoluble pellet samples from DC carcass halves compared to IC carcass halves was revealed (Table 4.6). Additionally, a trend ($P = 0.068$) toward increased M-protein in the insoluble pellet was observed for samples from RG carcasses compared to NG carcasses (Table 4.5). At this time, it is unknown if the decrease in extractability of M-protein in RG and DC turkey breast samples influenced the extractability of other myofibrillar proteins.

4.3.1.2.4 TITIN AND NEBULIN

Titin and nebulin are extremely large cytoskeletal proteins (approximately 3000 and 600 to 900 kDa, respectively; Huff-Lonergen *et al.*, 1996). Degradation of either titin or nebulin could result in protein fragments of substantial size; however,

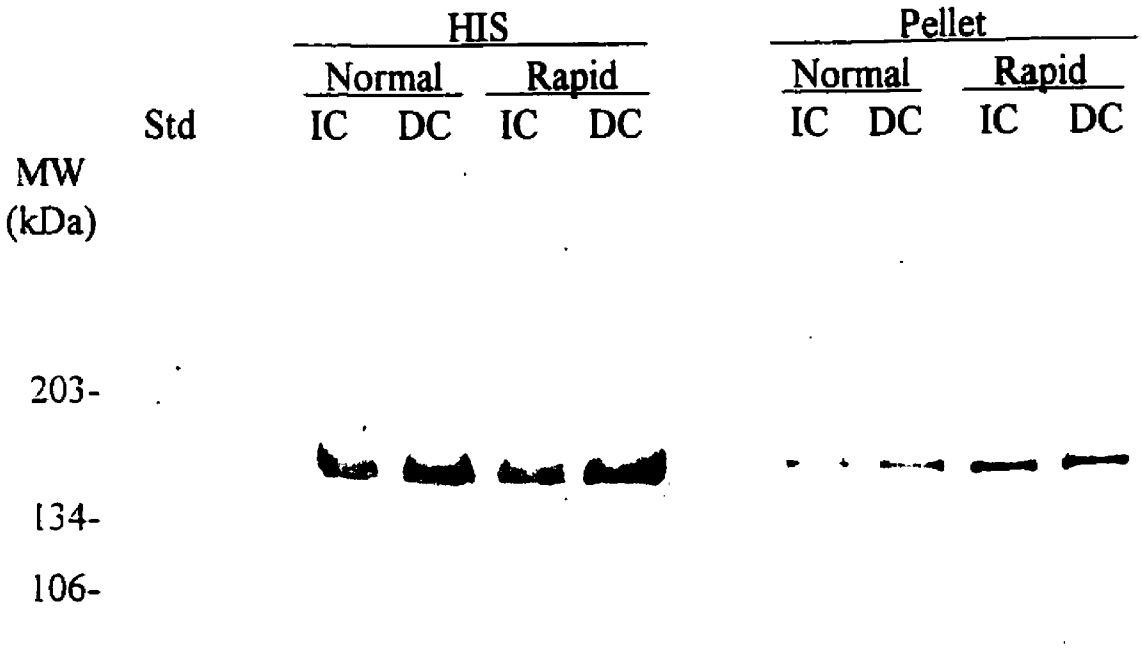

Figure 4.8 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 11% (100:1) gel, and probed with anti-M-protein. Each lane contains 20 μg of protein from extraction with high ionic strength (HIS) buffer or proteins insoluble in HIS (Pellet).

the 95, 133, 142, and 165 kDa protein bands identified with SDS-PAGE did not react with antibodies to either titin or nebulin. The antibody specific to titin reacted with the parent molecule (T1) and a very large degradation product known as T2 (Taylor *et al.*, 1995). A Western blot is not shown, however, the apparent location of T1 and T2 can be observed in the Coomassie stained SDS-PAGE gel in Figure 4.9. Large degradation products of nebulin were also detected by Western blotting (Figure 4.10). The bands associated with RG and DC samples in SDS-PAGE patterns did not react with the monoclonal antibodies developed against titin or nebulin. However, not all fragments of these large proteins will contain the epitopes that the antibodies against titin and nebulin recognize. Indeed, Ho *et al.* (1996) were able to identify numerous 39 to 130 kDa degradation products of titin using a monoclonal antibody different from that used in the current study.

In the present study, postmortem degradation of titin to its large fragment T2 was not affected by the rate of postmortem glycolysis or by the time chilling was initiated. This was determined by observation of T1 and T2 in myofibril samples on 5% (100:1) gels stained with Coomassie brilliant blue. These results do not support the reports of Pospiech *et al.* (1992), or Boles *et al.* (1992), who found decreased titin degradation for RG turkey breast samples and RG pork samples, respectively, compared to NG samples.

Degradation of nebulin was minimal in myofibril samples prepared from tissue frozen at 15 min and 3 h postmortem (Figure 4.9). However, anti-nebulin revealed increased degradation of nebulin in RG samples compared to NG samples by 24 h postmortem (Figure 4.9 and 4.10, Table 4.8). However, delayed chilling did not

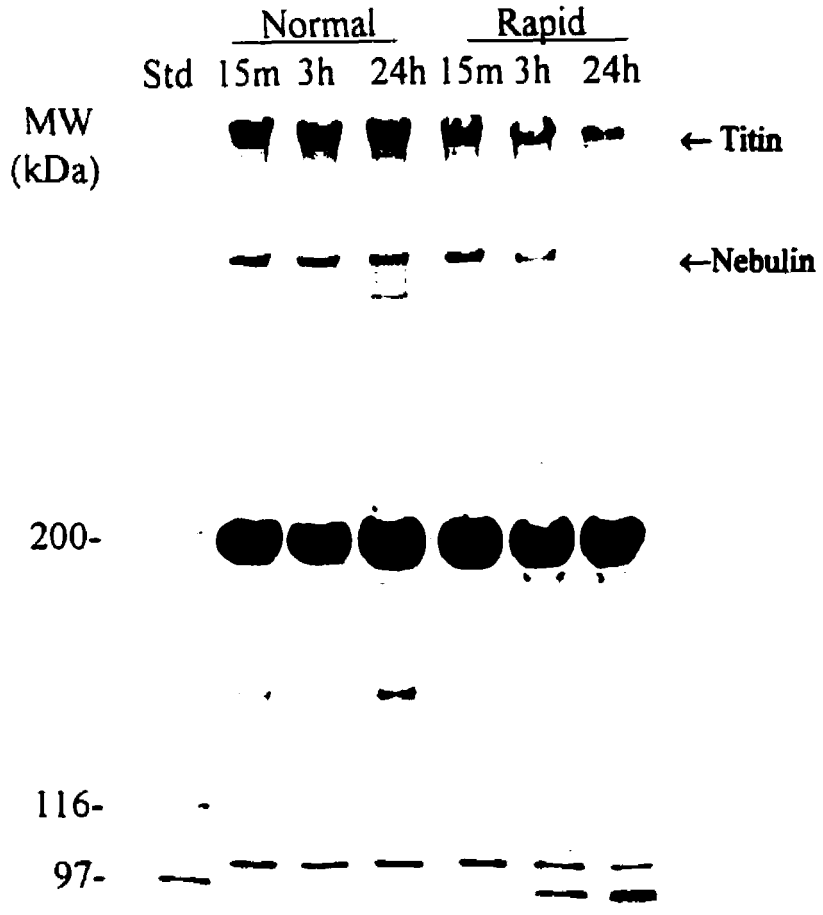

Figure 4.9 - SDS-PAGE patterns of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcasses on a 5% (100:1) gel stained with Coomassie brilliant blue. Each lane contains 20 μg of protein from washed myofibrils prepared from samples collected 15 min, 3 h, and 24 h postmortem.

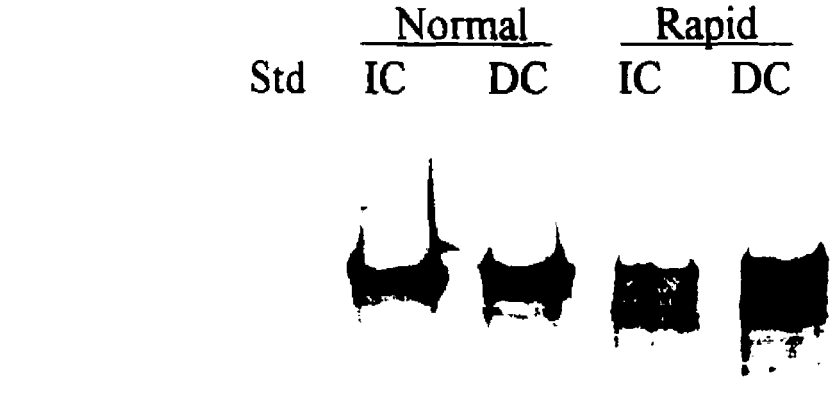

Figure 4.10 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from a 7% (100:1) gel, and probed with anti-nebulin. Each lane contains 20 μg of protein from 24 h postmortem myofibril preparations.

Table 4.8 - Arbitrary peak area values from densitometry of Western blots for nebulin and desmin from normal and rapid glycolyzing turkey breast and the effect of delaying carcass chilling

	Nebulin in Myofibril Preparations	Desmin in Myofibril Preparations
NG[1]	376	214
RG[2]	234	199
SEM	60 (n=4)	63 (n=2)
IC[3]	303	207
DC[4]	308	207
SEM	43 (n=8)	31 (n=4)

[1] NG = Normal Glycolyzing (pH > 6.00 at 15 min postmortem).
[2] RG = Rapid Glycolyzing (pH $\leq$ 5.80 at 15 min postmortem).
[3] IC = Immediately chilled (20 min postmortem).
[4] DC = Delay chilled (110 min postmortem).

affect the degradation of nebulin. Therefore, the effect of nebulin degradation on protein extractability is unclear since the rate of glycolysis and delay chilling were not additive with respect to nebulin degradation as they were with protein extractability. On average, densitometry results indicated 58% of nebulin in rapid glycolyzing samples was degraded compared to 33% in normal glycolyzing controls. Other investigators have reported increased degradation of nebulin in PSE pork (Warner *et al.*, 1997). However, Boles *et al.* (1992) did not find increased nebulin degradation for myofibrils prepared from RG pork as determined by SDS-PAGE analysis.

Discrepancies between the results of this study and results of other investigators for titin and nebulin degradation may be due to differences in the ultimate pH of samples. Watanabe and Devine (1996) reported that the rates of degradation of titin and nebulin were slowest when the ultimate pH of muscle was between 6.0 and 6.3 and that the rate of degradation of these two proteins decreased at pH values above or below this range. Carcasses in the current study were selected with similar ultimate pH values to observe the effect of the rate of pH decline postmortem on individual muscle proteins while minimizing the influence of different ultimate pH values.

4.3.1.2.5 MYOSIN HEAVY CHAIN

Myosin heavy chain (MHC) is a 200 kDa subunit of myosin (Miller *et al.*, 1989). Two monoclonal antibodies to MHC were used to probe for fragments of myosin in samples of transferred proteins. One antibody (F59) was specific for an

epitope located on the heavy meromyosin proteolytic fragment of MHC (Miller *et al.*, 1989). Fragments of MHC that reacted with clone F59 included 63, 71, 73, 84, 93, 95, 103 and 104 kDa (Figure 4.11). Interestingly, one of the MHC fragments was the same molecular weight as the band that reacted with anti-glycogen phosphorylase (95kDa). Thus, in other studies where Western blotting for glycogen phosphorylase was not performed, this band commonly designated glycogen phosphorylase may also be a fragment of MHC.

The second anti-MHC antibody, F27, which recognizes an epitope on the light meromyosin fragment (Miller *et al.*, 1989), labeled a number of polypeptides migrating at 133, 142, 152, 165, 175, and 189 kDa in the HIS and insoluble pellet fractions (Figure 4.12). These were atypical fragments of MHC degradation compared to proteolysis of MHC by trypsin, chymotrypsin, or papain (Pearson and Young, 1989). The samples positive for MHC fragments that reacted with F27 also had fragments that reacted with F59 and vice versa. F59 and F27 did not react with bands from any LIS-extracted samples and reacted only weakly for HIS and insoluble pellet fractions from NG/IC carcass halves. Similar weak responses were found for the HIS and insoluble pellet fractions of some NG/DC carcass halves (Figure 4.11), however, strong reactions for samples from other NG/DC carcass halves occurred (Figure 4.12). MHC fragments from the HIS and insoluble pellet fractions of RG carcasses reacted strongly with both MHC antibodies, and delayed chilling increased the intensity of these bands. The Western blot results with these two antibodies suggest that the altered SDS-PAGE banding patterns discussed earlier, are partly due to MHC degradation in RG and DC breast muscle.

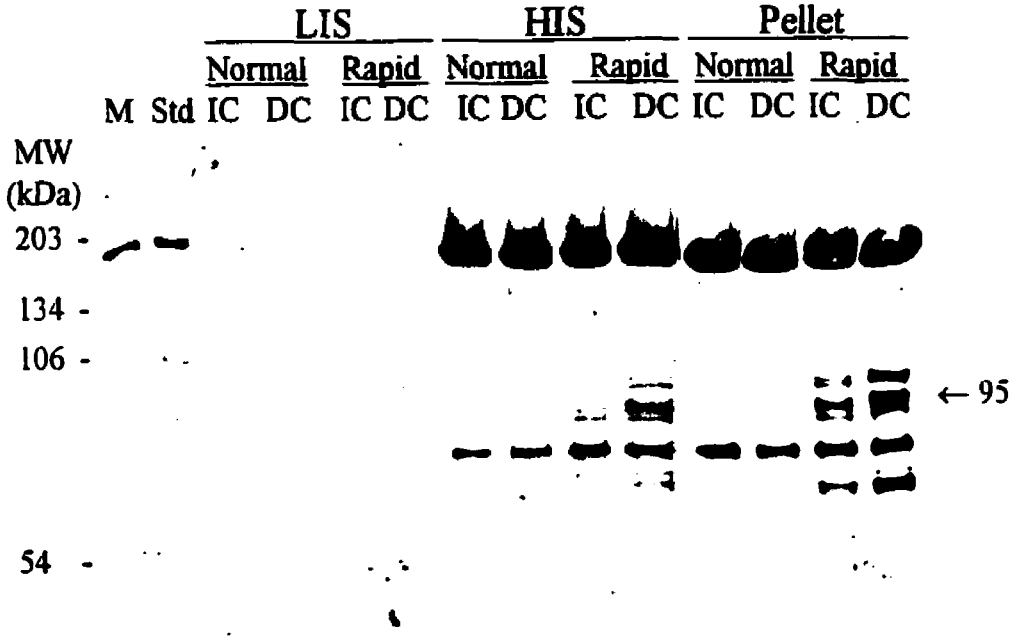

Figure 4.11 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from a 11% (100:1) gel, and probed with anti-heavy meromyosin (clone F59). Each lane contains 20 μg of protein from extraction with low ionic strength (LIS) buffer, high ionic strength (HIS) buffer, or proteins insoluble in HIS (Pellet).

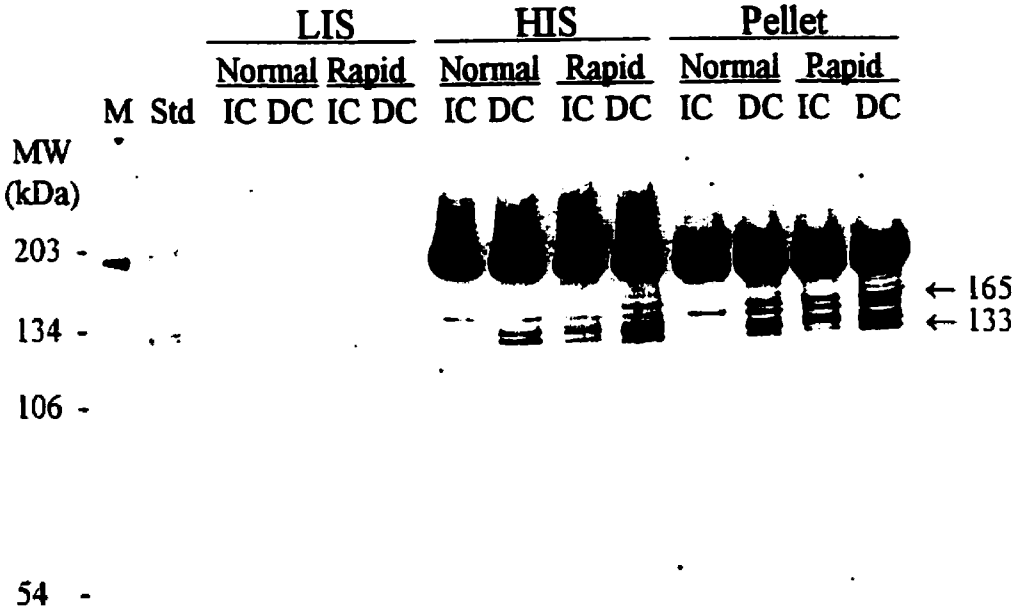

Figure 4.12 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 11% (100:1) gel, and probed with anti-light meromyosin (clone F27). Each lane contains 20 μg of protein from extraction with low ionic strength (LIS) buffer, high ionic strength (HIS) buffer, or proteins insoluble in HIS (Pellet).

SDS-PAGE analysis is often used to determine changes in extractability of proteins and/or to observe degradation of isolated proteins of interest. Densitometry of Coomassie brilliant blue stained gels for HIS-extracted proteins indicated there was a trend ($P = 0.07$) for reduction of the 200 kDa band (MHC) in DC samples. This reduction in the intensity of the 200 kDa band could be due to the degradation and/or loss of extractability of MHC. Aside from MHC and bands containing MHC fragments, the relative proportion of the majority of myofibrillar proteins extracted remained the same even when overall extractability was drastically reduced as in RG/DC samples. This suggests that the extractability of myofibrillar proteins as a whole was reduced. These findings are similar to those of Camou and Sebranek (1991), who found no difference in SDS-PAGE banding patterns for salt-soluble protein extracted from PSE and normal pigs.

Results from this study emphasize some of the problems in using SDS-PAGE to identify differences between samples. Any number of proteins and protein fragments with similar molecular weights can migrate to the same position in a gel. For instance, it was found that glycogen phosphorylase and a fragment of MHC were both located in a 95 kDa band. Additionally, proteins may be present below the detection limit of Coomassie brilliant blue staining. Thus, SDS-PAGE in combination with Western blotting provides improved detection and identification of proteins. Determining the extent of MHC degradation with SDS-PAGE would not be possible without the use of antibodies to confirm the presence and size of MHC fragments. To roughly determine the magnitude of MHC degradation in RG and DC

samples, washed myofibril samples, which contain both extractable and insoluble MHC, were probed with both F59 and F27. Figure 4.13 illustrates the difference in degradation of MHC in washed myofibril samples from RG and NG carcasses as well as for DC and IC carcass halves. The best estimate of degradation in RG/DC samples was 15 to 20% of the MHC. However, absolute numbers for the quantity of MHC degraded are difficult to attain due to problems inherent in using Western blotting for quantification (Towbin *et al.*, 1979; Jones *et al.*, 1995; Werner and Nagel, 1997). Regardless of the accuracy of this estimate, myosin degradation in RG/DC turkey breast muscle was noticeably greater than in NG/IC samples.

One might expect that degradation of a protein into smaller pieces may improve the extractability of the protein. Smith and Brekke (1985) found that proteolytic degradation of mechanically deboned fowl myofibrillar proteins by a commercial acid protease increased protein extractability. However, the sites of cleavage may be a very important factor influencing the extractability of myosin fragments. Heavy meromyosin is soluble at lower ionic strengths than intact MHC (Pearson and Young, 1989). The helical tail or rod in the light meromyosin fragment requires higher ionic strength solutions for solubilization (Lowey, 1971; Morrissey *et al.*, 1987; Pearson and Young, 1989). The presence of MHC fragments containing a large portion of the less soluble rod may influence the overall extractability of the myofibrillar proteins. Myosin is one of the least stable myofibrillar proteins at elevated temperatures and low pH values (Wright and Wilding, 1984). Therefore, denaturation and/or aggregation of myosin with other proteins may also reduce

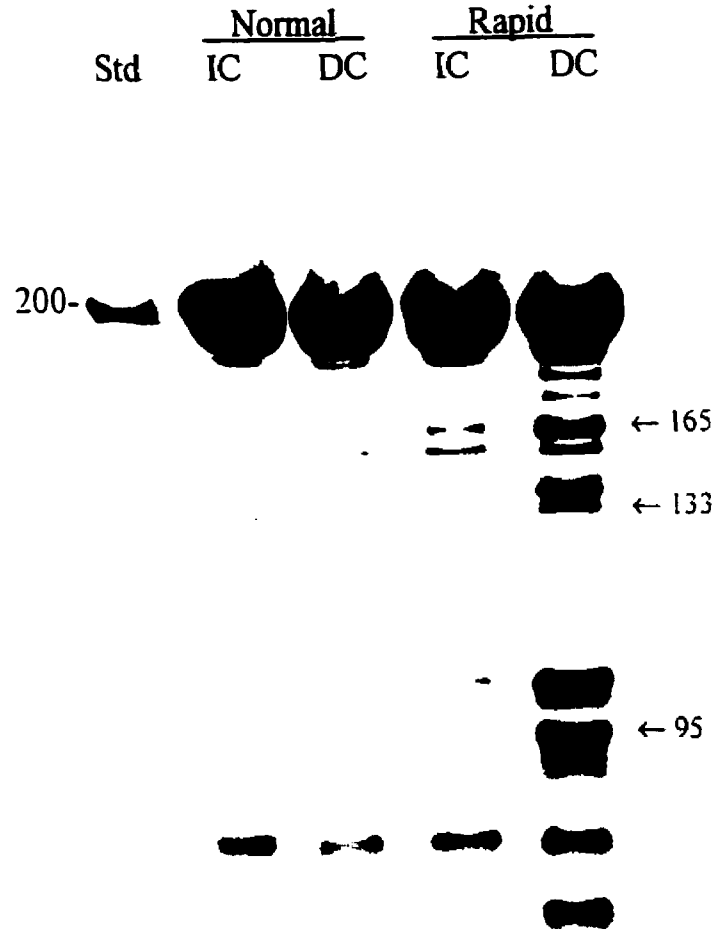

Figure 4.13 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 11 % (100:1) gel, and probed with both anti-heavy meromyosin (clone F59) and anti-light meromyosin (clone F27). Each lane contains 20 μg of protein from 24 h postmortem myofibril preparations.

myofibrillar protein extractability. The intensity of the reaction of antibodies with MHC fragments in the insoluble pellet appeared to be stronger than in the HIS fraction especially for the fragments larger than 152 kDa. This suggests that there is a reduction in extractability of some of the MHC fragments. However, the problems encountered with quantification of these bands makes this observation difficult to confirm.

O'Halloran *et al.* (1997) reported that both μ-calpain activity and release of cathepsins B and L are increased in RG bovine muscle compared to normal controls. Calpains have been implicated in the postmortem degradation of titin, nebulin, and Z-line proteins, including desmin (Taylor *et al.*, 1995). However, postmortem degradation of myosin is usually attributed to cathepsin activity (Zeece and Katoh, 1989; Whipple and Koohmaraie, 1991; Matsuishi *et al.*, 1992; Jiang *et al.*, 1996), which is optimum at temperatures near 37°C and low pH (4.0 to 6.0) values (Greaser, 1986). In the current study, myosin degradation was evident in samples collected 3 h postmortem (Figure 4.9) and increased by delay chilling when carcass temperatures would be elevated. However, nebulin degradation was minimal in 3 h postmortem samples and not increased by delay chilling. This suggests that myosin and nebulin are degraded at different rates by the same protease and/or that different proteases are involved in the degradation of each protein.

The presence of additional proteins (133 and 142 kDa) detected by SDS-PAGE analysis has not been reported previously for either PSE pork or RG turkey breast. However, proteolysis has been reported for postrigor meat samples held at the optimum pH and temperature for cathepsin activity. Yamamoto *et al.* (1979)

speculated that additional bands between 140 and 200 kDa in SDS-PAGE gels could be signs of catheptic degradation of myosin for postrigor chicken *P. major* homogenates adjusted to pH 5.5 and heated to 40°C for 6 to 24 h. Pospiech *et al.* (1997) found additional bands at ~132 and ~140 kDa for SDS-PAGE of porcine muscle samples exposed to elevated conditioning temperatures postmortem. Bandman and Zdanis (1988) found a single proteolytic fragment reacted with an anti-myosin antibody at ~125 kDa in samples from bovine muscle heated to 37°C for up to 3 days. Degradation by cathepsins was implicated because storage of the meat at 4°C, a temperature at which cathepsins are inactive, did not result in production of myosin fragments.

4.3.1.2.6 DESMIN

Desmin (~ 55 kDa) is a structural protein located in the Z-line region of the myofibril that binds adjacent muscle fibers together and is reported to be highly susceptible to proteolysis postmortem (Pearson and Young, 1989). Postmortem degradation of desmin by calpains is thought to be important with regard to postmortem tenderization of meat (Pearson and Young, 1989). Western blot results indicated that desmin was not extracted by either LIS or HIS as it was present only in the insoluble pellet samples (Figure 4.14). Additionally, there were no fragments of desmin detected in any of the samples. This does not necessarily mean there was no desmin degradation, because fragments of desmin may not contain the epitope for the monoclonal antibody to desmin. However, densitometry results of the band that

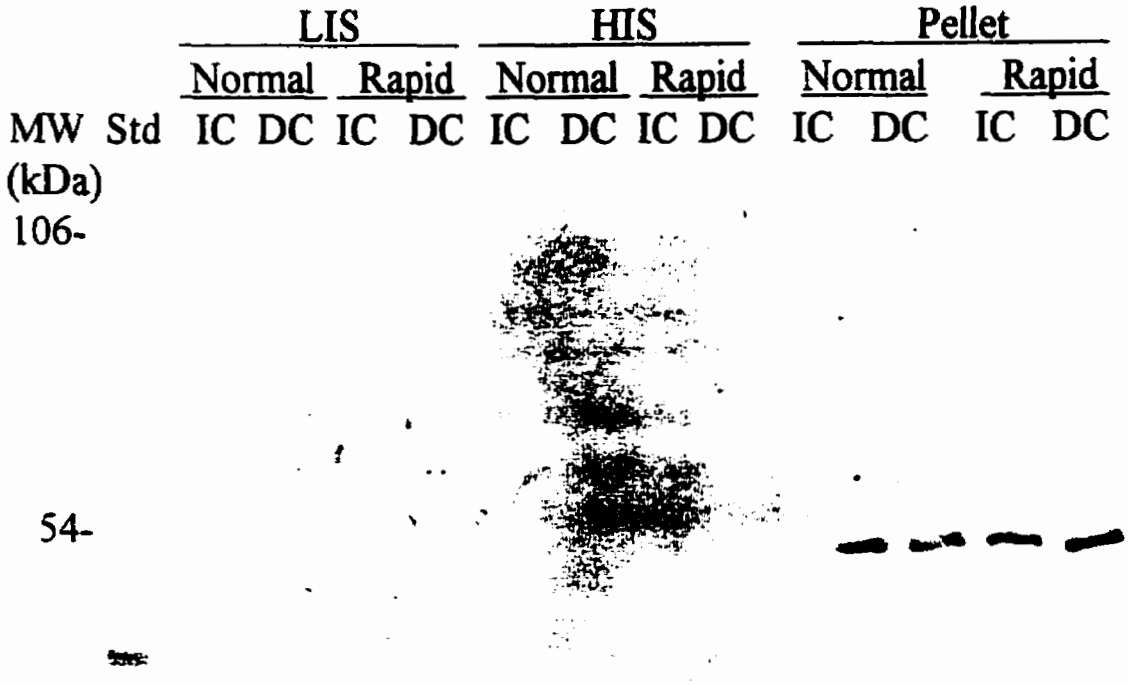

Figure 4.14 - A Western blot of turkey breast muscle proteins from normal and rapid glycolyzing turkey carcass halves chilled immediately (IC) or delay chilled (DC), transferred from an 11% (100:1) gel, and probed with anti-desmin. Each lane contains 20 μg of protein from extraction with low ionic strength (LIS) buffer, high ionic strength (HIS) buffer, or proteins insoluble in HIS (Pellet).

was detected showed there was no difference in the amount of this band for RG and NG samples nor for DC and IC samples (Table 4.8). Therefore, postmortem degradation of desmin was not effected by the rate of postmortem glycolysis or delay chilling of the turkey carcasses. These results are somewhat surprising in light of reports of extensive postmortem degradation of desmin in mammalian species (Pearson and Young, 1989).

4.3.2 POSTRIGOR HEATING

SDS-PAGE was used to screen for differences between the proteins in centrifuged samples compared to filtered samples following postrigor heating of turkey breast meat samples. Obvious differences in banding patterns were observed for the proteins present in LIS extraction buffer subjected to filtration compared to centrifugation (Figure 4.15). In particular, a 200 kDa myofibrillar protein present in the LIS of filtered samples was absent in the centrifuged samples. This indicated filtration did not separate myofibrillar proteins from sarcoplasmic proteins in the same manner as centrifugation.

Western blot analysis of these proteins with anti-myosin heavy chain antibodies illustrated that there was a significant amount of MHC in the LIS filtrate (Figure 4.16). The labeling of transferred proteins with anti-myosin heavy chain antibodies confirmed that the filtration method failed to stop insoluble protein from passing into the filtrate. In contrast, the 200 kDa protein was absent in LIS samples following centrifugation. Additionally, Western blot analysis of the precipitate from

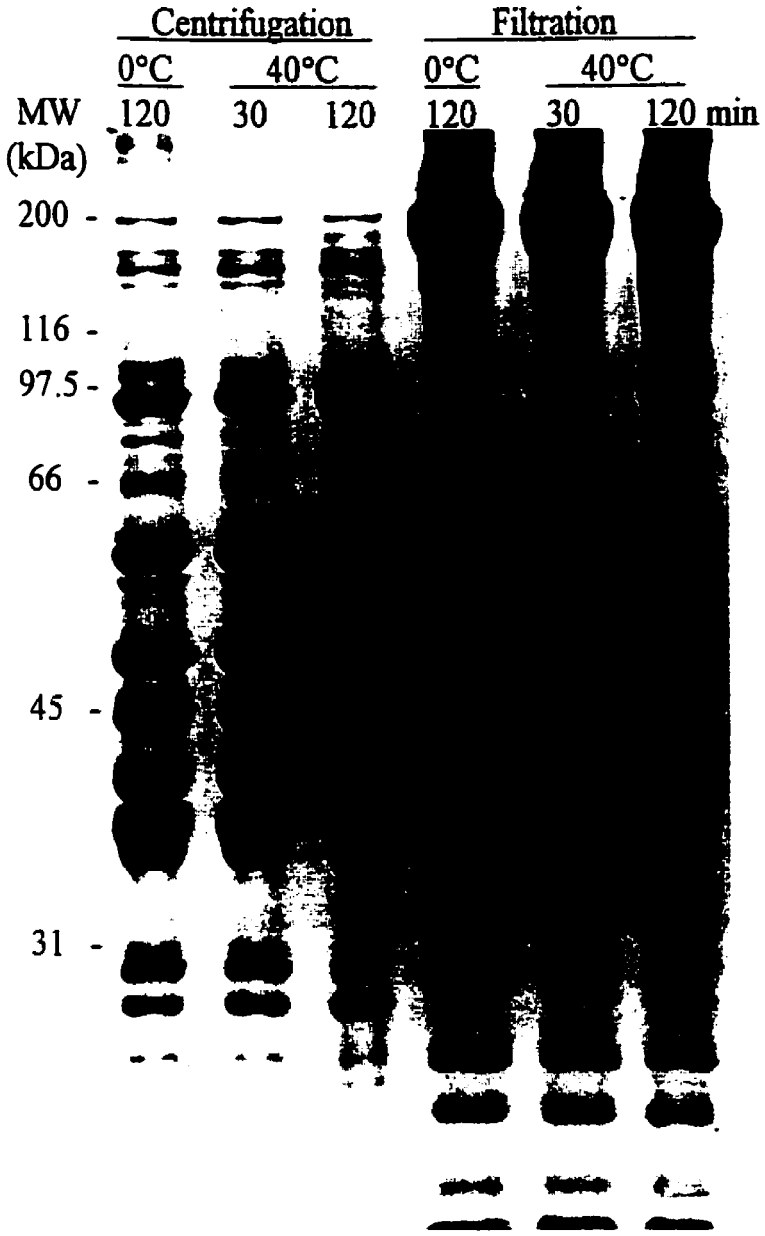

Figure 4.15 - SDS-PAGE pattern of postrigor turkey breast muscle proteins held at 0 or 40°C and extracted in low ionic strength buffer by filtration or centrifugation. The proteins were separated on a 10% (37.5:1) gel stained with Coomassie brilliant blue. Twenty-five micrograms of protein were loaded for each sample.

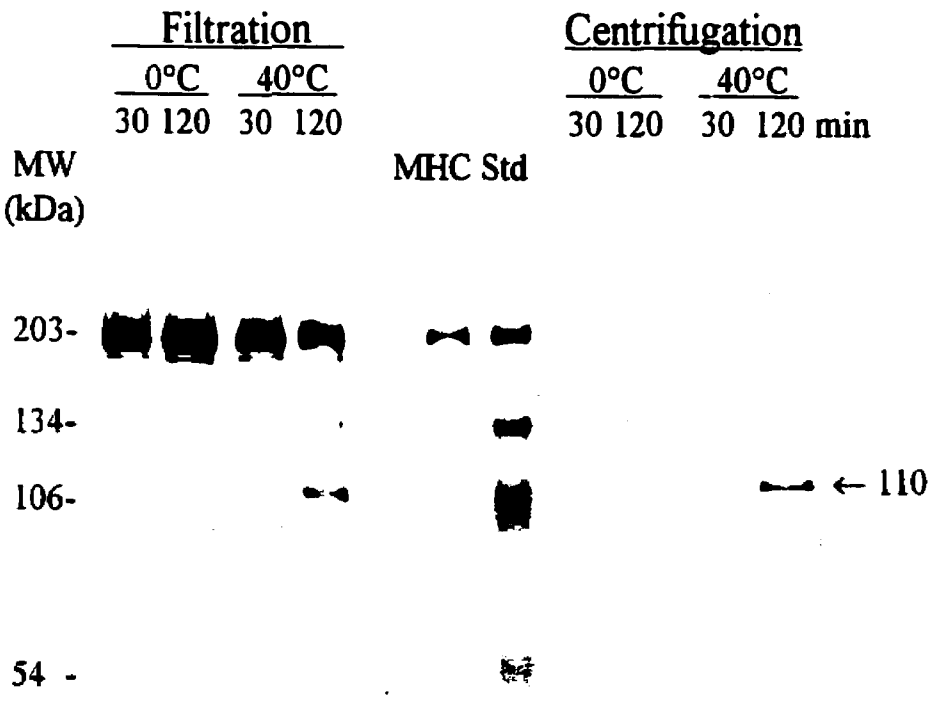

Figure 4.16 - A Western blot of postrigor turkey breast proteins held at 0 or 40°C, extracted in low ionic strength buffer by filtration or centrifugation, transferred from an 11% (100:1) gel, and probed with anti-myosin heavy chain antibodies. Lanes labeled MHC were loaded with myosin heavy chain standard. Lanes labeled Std were loaded with prestained molecular weight standards.

filtered samples extracted in LIS buffer and stored 48 h indicated that MHC was a substantial component of the precipitate (Figure 4.17).

SDS-PAGE analysis in combination with Western Blotting was employed to investigate changes to myosin following postrigor heating. The intensity of the 200 kDa band for MHC extracted in HIS buffer was reduced for samples from meat exposed to 40°C for 120 min compared to controls at held at 0°C. This suggested that less myosin was extracted upon exposure to 40°C postrigor. Labeling transferred proteins extracted in HIS from meat held at 40°C for 120 min with anti-MHC revealed extensive degradation of MHC compared to unheated samples (Figure 4.18). Fragments of MHC of 189, 152, 142, 133, 110, 104, 95, 84, 73, and71 kDa would account for the reduction in the intensity of the 200 kDa band with SDS-PAGE. Only one fragment of MHC (~110 kDa) was present in the supernatant of LIS extracted samples, specifically for samples held at 40°C for 120 min (Figure 4.16). The 110 kDa fragment reacted with monoclonal F59 which is specific to an epitope on heavy meromyosin, a proteolytic fragment of MHC (Miller *et al.*, 1989). This is consistent with reports that heavy meromyosin is soluble at low ionic strengths and intact MHC is insoluble in LIS buffer (Lowey, 1971; Pearson and Young, 1989).

In comparison to postrigor heated turkey breast samples the 110 kDa MHC fragment extracted in LIS was not present in the RG and DC turkey breast samples in the rigor development study (Figure 4.11). Myosin heavy chain fragments of 189, 152, 142, 133, 104, 95, 84, 73, and 71 kDa were observed in both the postrigor heating study and the RG and DC samples of the rigor development study.

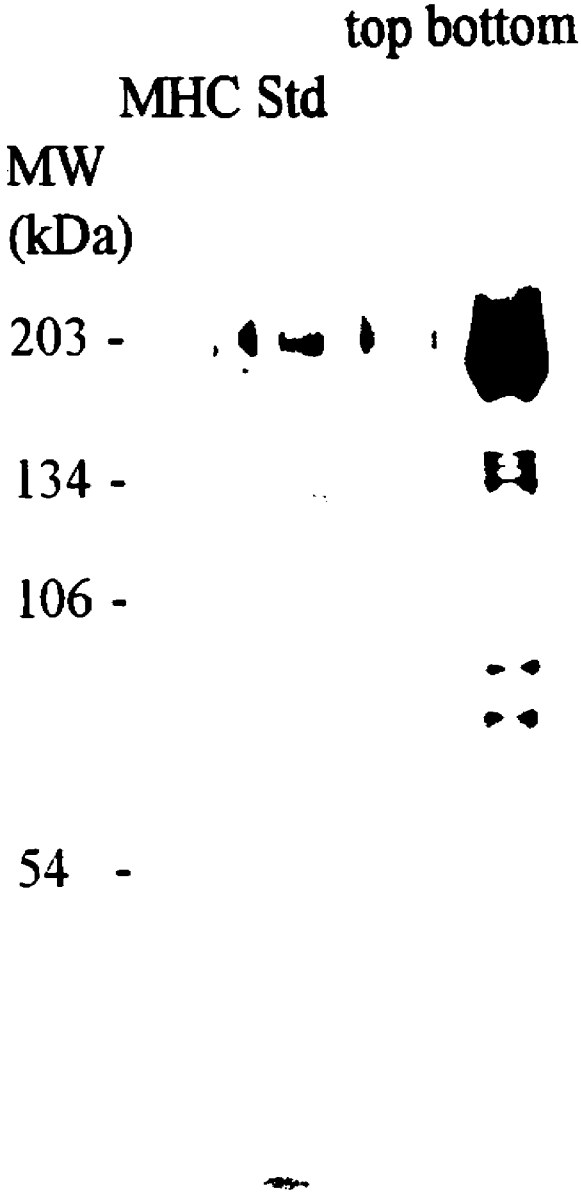

Figure 4.17 - A Western blot of postrigor turkey breast proteins held at 0°C for 120 min, extracted in low ionic strength buffer by filtration, transferred from an 11% (100:1) gel, and probed with anti-myosin heavy chain antibodies. Protein samples were held at 4°C for 48 h and a portion was removed from the top and bottom of the filtrate. Ten micrograms of protein was loaded for each sample. Lane labeled MHC was loaded with myosin heavy chain standard. Lane labeled Std was loaded with prestained molecular weight standards.

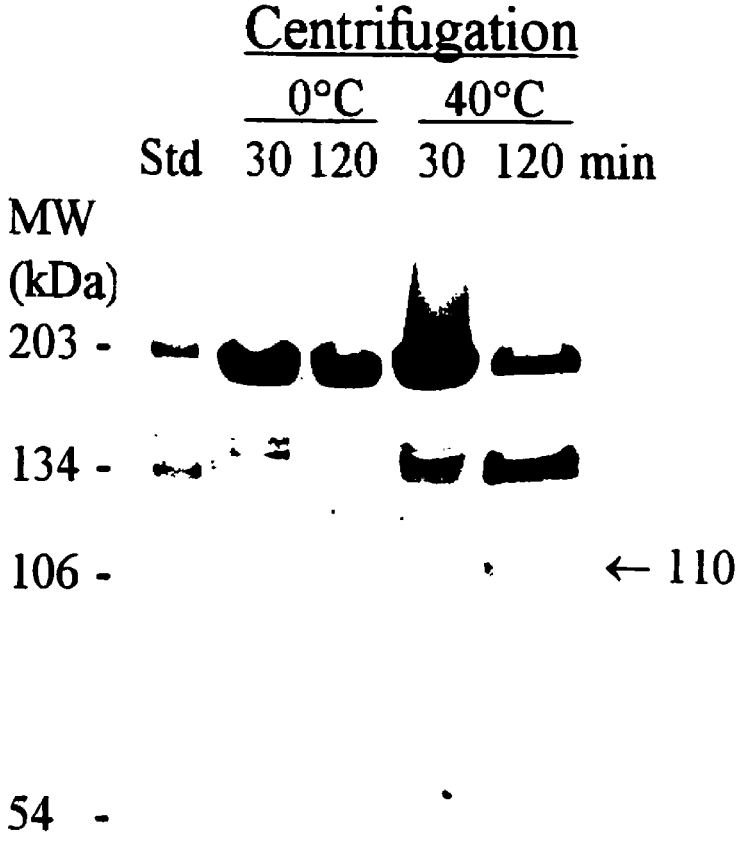

Figure 4.18 - A Western blot of postrigor turkey breast proteins held at 0 or 40°C, extracted in high ionic strength buffer by centrifugation, transferred from an 11% (100:1) gel, and probed with anti-myosin heavy chain antibodies. Ten micrograms of protein was loaded for each sample. Lane labeled Std was loaded with prestained molecular weight standards.

However, the 175, and 165, 103, 93, and 63 kDa MHC fragments in the RG and DC samples of the rigor development study were not found in postrigor heated samples. Birds from the postrigor heating trial were not selected based on 15 min postmortem pH and the breast muscle was frozen prior to the heating trial. Therefore, to ensure freezing and lack of carcass selection based on the rate of postmortem pH decline were not responsible for the altered pattern of MHC fragments. A separate trip to the commercial slaughter facility was made to select two additional NG and RG carcasses based on 15 min postmortem breast muscle pH. Breast samples from these carcasses were heated postrigor without a freezing step. Figure 4.19 shows that MHC degradation in washed myofibril samples from RG breast meat and fresh NG postrigor breast meat held at 40°C for 90 min have the same differences in banding patterns as reported in the previous paragraph.

These differences in MHC degradation may indicate that postrigor heating is inappropriate as a model for PSE-like turkey breast meat. The differences in MHC degradation in the postrigor heating study compared to the rigor development study may be due to the extent of MHC degradation in postrigor heated samples compared to unheated RG samples or differences in the activity of individual endogenous proteases. In addition to differences in MHC degradation, the extractability of both the sarcoplasmic proteins and myofibrillar proteins was reduced to a greater extent in postrigor heated samples (83% and 46% of unheated, respectively) compared to the reduction due to rapid postmortem glycolysis (93% and 66% of NG).

Figure 4.19 - A Western blot of postrigor breast myofibrillar proteins from a normal glycolyzing turkey held at 0 or 40°C and breast myofibrillar proteins from a rapid glycolyzing turkey held at 0°C, transferred from an 11% (100:1) gel, and probed with anti-myosin heavy chain antibodies. Ten micrograms of protein was loaded for each sample. The lane labeled Std was loaded with myosin heavy chain.

5. SUMMARY AND CONCLUSIONS

Several controlled studies were conducted to evaluate the role of postmortem muscle pH and carcass temperature on turkey breast meat quality. Initially the experimental emphasis was to determine the changes in protein extractability of postmortem turkey breast exposed to conditions of low pH and physiological temperatures using the heating of postrigor turkey breast to 40°C as a model. Fischer *et al.* (1979) developed this model for producing PSE-like pork characteristics by heating postrigor pork to 40°C for 90 min. Changes in protein extractability were monitored because extractability is an important functional property of muscle proteins often used as an indicator of the potential value of meat for processed products (Morrissey *et al.*, 1987). Lopez-Bote *et al.* (1989), Boles *et al.* (1992), van Laack *et al.* (1993), and Warner *et al.* (1997) reported that the extractability of proteins in PSE pork is much lower than for normal pork. Heating postrigor turkey breast to 40°C caused substantial reductions in extractability of both sarcoplasmic and myofibrillar proteins. Extractability of the myofibrillar proteins was reduced to a greater extent than sarcoplasmic proteins.

In the postrigor heating study it was determined that not all methods for determining protein extractability are suitable for this type of study. There was more variability in protein extractability measurements using the filtration method compared to the method utilizing centrifugation to separate extractable proteins. A

certain amount of insoluble protein was not effectively removed in the filtration procedure. This was especially evident when LIS extraction solution was used to extract the sarcoplasmic proteins as indicated by the presence of MHC in the Western blots of these samples and in the precipitate after 48 h of refrigerated storage.

Changes to protein extractability of postrigor turkey breast meat following heating was evaluated, however, the intended use for postrigor heating was as a model to mimic changes that occur when development of rigor mortis is rapid. It became difficult to determine if postrigor heating was a suitable model due to the lack of knowledge with respect to changes in turkey breast meat quality due to rapid postmortem glycolysis. Therefore, additional studies were conducted to establish the influence of rapid postmortem glycolysis in turkey breast muscle and a delay in chilling on breast meat quality. Carcasses were categorized based on early postmortem breast muscle pH and comparisons were made between carcasses with similar ultimate pH values. Analysis of colour measurements on ground breast meat samples indicated that rapid postmortem glycolysis did not result in increased paleness. At first glance these results appear to contradict evidence from studies where increased paleness for rapid glycolyzing turkey breast meat was reported. However, it must be pointed out that because carcasses were selected with similar ultimate pH values the samples in this study may not have been from a normally distributed population. Even though colour differences were not observed, rapid postmortem glycolysis reduced both sarcoplasmic and myofibrillar protein extractability, and the strain at fracture for cooked meat gels. If rapid postmortem

glycolysis results in a decrease in ultimate pH these effects would likely be exacerbated.

In addition to selecting carcasses based on 15 min postmortem breast muscle pH, one half of each turkey carcass was held prior to immersion chilling in an insulated container. This operation was performed in order to increase the likelihood that breast muscle proteins would be exposed to conditions of high temperature and low muscle pH. It was thought that a delay in immersion chilling may increase the magnitude of any changes to breast meat quality thereby facilitating the detection of otherwise small changes in quality. Delay chilling was found to reduce breast meat quality with no significant interaction with the rate of postmortem glycolysis suggesting that the negative effects on quality were additive for rapid postmortem glycolysis and delay chilling. These negative effects of delay chilling included a reduction in sarcoplasmic and myofibrillar protein extractability, reduced cook yield, and reduced stress and strain at fracture for cooked meat gels. There was a meaningful correlation between myofibrillar protein extractability and cook yield and strain at fracture for breast meat gels. This relationship between myofibrillar protein extractability and important meat quality measurements indicates that protein extractability measurements can provide very useful information.

Once it was established that rapid postmortem glycolysis and delay chilling were associated with reductions in turkey breast meat quality, the next step was to identify individual proteins affected by the rate of postmortem glycolysis and delay chilling. The postmortem changes to muscle proteins from rapid glycolyzing meat are not well understood. Reduced protein extractability in PSE pork has been

attributed to denaturation of both sarcoplasmic and myofibrillar proteins, particularly myosin (Stabursvik *et al.*, 1985; Offer, 1991). Additionally, rapid postmortem glycolysis has been reported to influence the postmortem degradation of titin and nebulin, which are large structural proteins of the myofibril.

SDS-PAGE in combination with Western blot analysis was used to determine which proteins were altered by differences in the rate of postmortem pH decline and delayed chilling. A systematic approach was used to identify proteins involved in changes to SDS-PAGE banding patterns. Normally, investigators would extract proteins with a high ionic strength solution and perform SDS-PAGE in an attempt to determine which proteins had reduced extractability. However, in this case SDS-PAGE was performed on proteins extracted in both low and high ionic strength solutions as well as the proteins not extracted by either solution. The analysis of the insoluble fraction was very useful for identifying changes to protein samples.

The identification of several proteins using immunoblotting revealed some novel information regarding changes to muscle proteins in rapid glycolyzing and delay chilled turkey breast. These changes include, a reduction in the extractability of the sarcoplasmic proteins glycogen phosphorylase and creatine kinase and a reduction in the extractability of M-protein. Bendall and Wismer-Pedersen (1962) speculated that precipitation of sarcoplasmic proteins reduced the extractability of the myofibrillar proteins in PSE pork. In this study there were increased amounts of both glycogen phosphorylase and creatine kinase from RG and DC turkey breast that were not extracted by low ionic strength buffer. However, not all sarcoplasmic proteins were affected in this way as evidenced by the detection of glycogen

debranching enzyme in only the low ionic extracts. The reduction in M-protein extractability may influence the extractability of other myofibrillar proteins as it is a key structural protein of the myofibril.

Perhaps the most notable change due to rapid postmortem glycolysis was the increased degradation of both nebulin and myosin in the breast meat of RG carcasses. Nebulin degradation occurred after 3 h postmortem and was not influenced by delay chilling suggesting that nebulin degradation does not contribute to reduced protein extractability, which is observed for both RG and DC samples. However, myosin degradation occurred early postmortem and was increased by a delay in chilling. The association of myosin degradation with samples of reduced protein extractability indicated that degradation of myosin might play an important role in the development of PSE-like turkey breast. Until now there have been no reports of increased myosin degradation for rapid glycolyzing turkey breast or for PSE pork.

Not only was the presence of MHC degradation associated with RG and DC turkey breast with lower meat quality but the banding pattern of the fragments provided some additional information. The extractability and size of some of the MHC fragments from RG and DC turkey breast were different than fragments of MHC identified in postrigor heated breast meat. This indicates that postrigor MHC degradation may be different from degradation that occurs during the development of rigor. For this reason the postrigor heating model for PSE-like turkey breast may have limited applications.

One interesting finding from this study is that one of the fragments of MHC degradation in RG and DC turkey breast is the same size as glycogen phosphorylase.

Therefore, changes to the extractability of glycogen phosphorylase in PSE or PSE-like meat can not be monitored by SDS-PAGE alone as this protein can easily be confused with the MHC fragment.

MHC fragments from RG and DC appeared to be less extractable since there was a stronger reaction with myosin antibodies for fragments in the insoluble pellet than in extractable fractions. This observation suggests that myosin degradation may contribute to the observed reduction in protein extractability for RG and DC breast meat samples. The association of postmortem protein degradation with meat of reduced quality merits further investigations. It is difficult to determine if the degradation of myosin in RG and DC turkey breast meat is responsible for the reduction in meat quality or just associated with breast meat of poor quality. It has been established that myofibrillar proteins are denatured in PSE pork (Bendall and Wismer-Pedersen, 1962; Stabursvik *et al.*, 1984). A potential interaction of degradation and denaturation of MHC may have a significant impact on meat quality. The influence of postmortem degradation of myofibrillar proteins on turkey meat quality is an area of research that warrants further exploration as proteolysis of these proteins could potentially be responsible for costly losses in the meat industry.

The design of the experiments in this study made it possible to contribute new information to what is already established in the field of postmortem changes to turkey meat quality. Simple selection of turkey carcasses with similar ultimate pH values made it possible to determine that rapid postmortem glycolysis can reduce meat quality without necessarily changing ground breast meat colour. This is important information for investigators attempting to sort turkey breast meat of

different quality based on colour measurements. The act of dividing each turkey carcass into two halves made it possible to determine changes to meat quality within the one individual when carcass chilling is modified. This provided evidence that it is important for turkey processors to avoid delays in chilling of carcasses during the slaughter procedure in order to maintain breast meat quality.

Applications of existing biochemical techniques and refinement of some of these techniques were instrumental in making new discoveries throughout the course of this research. This is the first time a reduction in the extractability of glycogen phosphorylase and creatine kinase due to rapid postmortem glycolysis in turkey breast meat has been confirmed using statistical analysis of densitometry results from immunoblotting. Additionally, this is the first time that changes in the extractability of M-protein in turkey breast meat has been reported and confirmed by immunoblotting. Manipulation of the percentage of acrylamide in gels as well as the ratios with cross-linker led to the discovery of proteolytic fragments of MHC and changes in the degradation of nebulin.

The results of these studies indicate that the development of turkey breast with poor meat quality is not a simple issue but rather a complex problem that involves a number of proteins within the muscle cell. There are a number of approaches that could be taken to address this problem of PSE-like turkey breast meat. Optimally the goal of the turkey industry would be to find the cause of the problem and eliminate it. Alternatively, methods to identify low quality breast meat and divert it from production of processed products would be beneficial until a significant reduction in the incidence of PSE-like breast meat is realized. Another

possible approach is to modify further processing procedures to allow for salvage of diverted low quality breast meat.

If rapid postmortem glycolysis is the main cause of PSE-like turkey breast meat, factors which influence the rate of postmortem glycolysis need to be addressed. Stress during production and more importantly prior to slaughter can affect the rate of postmortem glycolysis. Avoidance of heat-stress during production may be of particular importance to the turkey industry (McKee and Sams, 1997). Stress to the birds during catching and loading at the farm and handling at the processing facility should be minimized. Monitoring bird stress may allow investigators to determine feasible changes to production procedures which will impact meat quality.

Modifications to slaughter procedures may also have an impact on the rate of postmortem glycolysis in turkey breast meat. Electric stunning of poultry reduces the rate of postmortem glycolysis compared to gas stunning (Kang and Sams, 1999). However, proponents of gas stunning cite the fact that gas stunned poultry have fewer broken bones and hemorrhages than electrically stunned birds (Kang and Sams, 1999). The rate of postmortem glycolysis can also be reduced by more rapid cooling of carcasses (Greaser, 1986). Therefore, reducing the time that carcasses are at temperatures that accelerate postmortem metabolism is simple way to minimize rapid postmortem glycolysis. Proper maintenance of processing equipment is important to avoid stoppages of the processing line which can delay initiation of the chilling process. Increasing the line speed so carcasses are chilled earlier postmortem or increasing the rate of temperature decline in the chiller are potential methods to

avoid stimulating rigor acceleration with high carcass temperatures. However, the rate of carcass chilling needs to be balanced to avoid the occurrence of increased meat toughness due to cold shortening of muscle (Sams, 1999).

The location of poor quality breast meat within the breast muscle may have a bearing on how this problem should be addressed. The large size of commercial turkey breast muscles may make it very difficult to cool the center of the muscle at an appropriate rate. Turkeys could be marketed at a younger age to reduce the size of the breast muscle and improve carcass chilling rates. However, larger birds provide increased returns on input costs required for processing.

An alternative to modifying processing procedures to influence the rate of postmortem glycolysis is to decrease the susceptibility of commercial turkeys to rapid postmortem glycolysis. Results of these studies indicate there is a percentage of the population of commercial turkey breast muscle that exhibits rapid postmortem glycolysis. It has been suggested that there may be an increased incidence of PSE-like breast meat in populations selected for growth rate and carcass size. Delay chilling in combination with screening for MHC degradation could be used to determine if some populations of turkeys are more prone to rapid postmortem glycolysis.

Information from this work may also have applications in the production of meat and meat products from other livestock species such as broiler chickens and pork. The degradation of MHC has not been reported in PSE pork. However, myofibrillar protein degradation studies may reveal new aspects of this meat quality problem that may lead to substantial cost savings for the meat industry in general.

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