Rapid Post-Mortem Glycolysis and Delay Chilling of Turkey Carcasses Cause Alterations to Protein Extractability and Degradation of Breast Muscle Proteins

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SDS−PAGE banding patterns of myofibrillar protein samples from turkey breast muscle with pH ≤5.8 at 15 min post-mortem (rapid glycolyzing) contained 133, 142, and 165 kDa bands that were absent in samples from carcasses with pH >6.0 at 15 min post-mortem (normal glycolyzing). These extra protein bands contained fragments of myosin as identified by Western blot analysis. Myosin fragments were also observed in protein samples from breast muscle not allowed to cool until 110 min post-mortem (delay chilled). In addition to myosin degradation, neublin degradation was more extensive in samples from rapid glycolyzing carcasses than for normal controls. Creatine kinase and glycogen phosphorylase were present in myofibrillar protein extracts of rapid glycolyzing carcasses in higher quantities than in normal controls. Results of this study provide insight into the molecular basis for previously reported reductions in meat quality of rapid glycolyzing and delay chilled turkey meat.

Keywords: *Turkey; post-mortem glycolysis; chilling; protein degradation*

INTRODUCTION

The rates of pH and temperature decline in post-mortem muscle influence the suitability of turkey breast meat for processed meat products. Cooked turkey breast products manufactured from meat with extremely rapid post-mortem glycolysis tend to have a coarse texture, fall apart, and exhibit excessive purge (McKee and Sams, 1998). Breast muscle from rapid glycolyzing (RG) turkeys has significantly reduced protein extractability (Pietrzak et al., 1997; Rathgeber et al., 1999), which was negatively correlated with cooking yield and cohesiveness of breast meat gels (Rathgeber et al., 1999).

Holding turkey carcasses at elevated temperatures early post-mortem can also influence breast meat quality (McKee and Sams, 1998; Rathgeber et al., 1999). McKee and Sams (1998) reported increased drip loss, cooking loss, and shear values for turkey breast placed in 40 °C water for 4 h immediately after evisceration compared to breast meat held in 20 and 0 °C water.

Rathgeber et al. (1999) investigated changes to protein extractability of RG turkey breast held at elevated temperatures early post-mortem. Myofibrillar protein extractability of turkey breast meat with pH ≤5.80 at 15 min post-mortem was reduced to 66% of values from breast meat with pH >6.00 at 15 min post-mortem. Holding carcasses at 40 °C until 110 min post-mortem prior to chilling reduced myofibrillar protein extractability of breast meat to 69% of values from carcasses chilled at 20 min post-mortem. When RG carcasses were held at 40 °C until 110 min post-mortem, protein extractability was 44% of normal glycolyzing (NG) carcasses chilled at 20 min post-mortem.

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Decades prior to the recognition that rapid post-mortem glycolysis was associated with meat defects in turkey breast, pork with pale, soft, and exudative (PSE) characteristics was reported as a significant meat quality problem (Bendall and Wismer-Pedersen, 1962). It is generally accepted that the combination of low muscle pH and high carcass temperature is responsible for alterations to proteins in PSE pork (Bendall and Wismer-Pedersen, 1962; Fischer et al., 1979; Stabursvik et al., 1984; Offer, 1991; Fernandez et al., 1994). 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). Bendall and Wismer-Pedersen (1962) hypothesized that sarcoplasmic proteins in PSE pork coated the myofibrils, thereby reducing the extractability of myofibrillar proteins. They observed that some sarcoplasmic proteins were not washed out of myofibrils from pork muscle that developed rigor at 37 °C. 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 post-mortem.

In addition to reduced extractability of myofibrillar and sarcoplasmic proteins, rapid post-mortem glycolysis has been reported to influence the post-mortem degradation of large structural proteins of the myofibril. Using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE), Boles et al. (1992) observed that titin was not degraded as rapidly in myofibrils from pigs genetically susceptible to stress as from normal pork. They suggested that maintaining the structural integrity of the myofibril may influence myofibrillar protein extractability.

The molecular basis for reduced meat quality associated with PSE pork is not fully understood. Even less

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is known about the post-mortem changes to turkey breast proteins exposed to low muscle pH and elevated carcass temperatures. Deterioration in functionality of turkey breast proteins may be best understood by observing changes to individual proteins. The objective of this study was to identify proteins affected by rapid post-mortem glycolysis or delayed chilling. The first step was to identify differences in SDS−PAGE banding patterns of breast protein samples from carcasses differing in rate of post-mortem glycolysis and chilled at different times post-mortem. The second step was to identify proteins involved in altered banding patterns by probing blotted samples with specific antibodies.

MATERIALS AND METHODS

Materials. Commercially slaughtered turkey carcasses weighing 10.5−12 kg were used. Potassium phosphate (mono and dibasic), KCl, EDTA, NaN_3, Tris, Triton X-100, $NaHCO_3$, and NaCl were obtained from BDH Inc. (Toronto, ON). Iodoacetate, bovine serum albumin, EGTA, phenylmethanesulfonyl flouride (PMSF), soybean trypsin inhibitor, benzylarginyl-methyl ester (BAME), Tween 20, *N*,*N*-dimethylformamide, clones T11 and NB1, and secondary antibodies conjugated to alkaline phosphatase (rabbit anti-goat, goat anti-rabbit, and goat anti-mouse) were obtained from Sigma (St. Louis, MO). Urea, thiourea, $MgCl_2$, and methanol were purchased from Fisher Scientific (Nepean, ON). Leupeptin and pepstatin were products of Calbiochem (San Diego, CA), and L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) was from ICN Pharmaceuticals Ltd. (Montreal, PQ). Acrylamide/*N*,*N*′-bis-(methylene acrylamide) solution, sodium dodecyl sulfate (SDS), nitrocellulose, 2-mercaptoethanol, bromophenol blue, Coomassie brilliant blue R-250, glycine, gelatin, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), and nitro blue tetrazolium (NBT) were purchased from Bio-Rad (Hercules, CA). The polyclonal antibodies to MM isozyme of creatine kinase and clone 10H5 were from Biodesign International (Kennebunk, ME). Clone 9063αDE was a gift from Dr. Y. T. Chen, Duke University (Durham, NC). Clone A6 was a gift from Dr. J. C. Perriard, Institute of Cell Biology (Zurich, Switzerland). Clones F27 and F59 were gifts from Dr. F. E. Stockdale, Stanford University (Palo Alto, CA). All reagents were of analytical grade.

Sample Selection and Preparation. Thirty-two tom turkey carcasses were selected from a commercial processing line at 15 min post-mortem. Carcasses with a 15 min post-mortem breast muscle pH ≤5.80 were classified as RG, and carcasses with a 15 min post-mortem pH >6.00 were classified as NG. Selection of turkey carcasses was performed on five separate days.

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 post-mortem, 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 post-mortem 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. 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.

At 18−24 h post-mortem, a 2−3 g sample of Pectoralis major was removed from an area next to the anterior point of the keel from each carcass half. 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 post-mortem. 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 18−24 h post-mortem 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 post-mortem, 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.

Muscle pH. The 15 min post-mortem breast muscle pH for on-line carcass selection was measured in triplicate using a spear tip pH electrode as described by Rathgeber et al. (1999). The pH of the samples frozen 15 min post-mortem 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 post-mortem, the ultimate pH of breast samples was determined using 20 g of tissue blended for 1 min in 80 mL of deionized water. The pH of the slurry was determined with a standard Accumet pH electrode (Accumet, Fisher Scientific, Nepean, ON). At this point, carcasses with similar breast muscle ultimate pH values were retained for further experimentation. The average ultimate pH of the retained RG samples was 5.68 compared to 5.71 for the NG samples. A total of 24 (12 RG and 12 NG) carcasses were retained for further analysis.

Sarcoplasmic and Myofibrillar Protein Preparation. The sarcoplasmic and myofibrillar proteins were extracted using a procedure described by Rathgeber et al. (1999). One-gram samples of ground breast meat were homogenized (Polytron PT 10/35, Brinkmann Instruments Canada Ltd., 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, 2 °C) for 10 s and placed on ice for 30 min. These samples were centrifuged at 17500*g* for 15 min at 2 °C. The supernatant was retained as the sarcoplasmic protein extract. The pellet was resuspended in an additional 20 mL of LIS buffer, homogenized, and centrifuged as described above. 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, 2 °C). Following centrifugation, the supernatant was retained as the myofibrillar protein extract.

The SDS−PAGE banding patterns for sarcoplasmic and salt soluble protein samples from all 48 carcass halves were examined. Additionally, the insoluble pellet following extraction with HIS from each half of 8 carcasses (4 RG and 4 NG from last slaughter date) was prepared for electrophoresis. The insoluble pellet was washed three times with 5 mM Tris-HCl (pH 8.0) and centrifuged at 1500*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% (w/v) SDS, 0.7 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 °C (Boles et al., 1992), and centrifuged for 3 min at 6600*g*.

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 all 3 and 24 h post-mortem samples as described by Goll (1974) and modified by Boles et al. (1992). Protease inhibitors were added to all buffers used in the wash procedure (0.1 mM PMSF, 2 mg/L leupeptin, 1 mg/L pepstatin, 10 mg/L TPCK, 10 mg/L soybean trypsin inhibitor, 10 mg/L BAME). Protein determination and preparation for SDS−PAGE of the washed myofibrils was as described for samples extracted in LIS and HIS buffer.

SDS−PAGE. SDS−PAGE was performed as outlined by Laemmli (1970) using a Mini-Protein II electrophoresis unit (Bio-Rad Laboratories). Protein samples analyzed included LIS and HIS extracts, residual proteins remaining after extraction by HIS, and the washed myofibril preparations. These samples were loaded at 20 μg of protein per lane on resolving gels of 11% [100:1 acrylamide/*N*,*N*′-bis(methylene acrylamide], 10% (75:1), 8% (100:1), or 7% (100:1) each with a 4% (37.5:1) stacking gel. Gels were run at 35 mA, 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, 10% glacial acetic acid, and 40% methanol. Gels were 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.

Immunoblotting. Following SDS−PAGE of samples from each half of eight carcasses (four RG and four NG from last slaughter date), the proteins were transferred to nitrocellulose membranes (0.45 μm, Bio-Rad Laboratories) at 40 V for 2.5 h using a Transblot Cell (Bio-Rad Laboratories). Samples probed for the larger proteins, titin and nebulin, were transferred at 40 V for 3 h followed by an additional 2.5 h at 45 V. The transfer buffer consisted of 25 mM Tris, 192 mM glycine, 0.1% SDS, and 20% (v/v) methanol (Towbin et al., 1979). Following transfer, the nitrocellulose was washed for 10 min in Tris-buffered saline (TBS, 20 mM Tris, 500 mM NaCl, pH 7.5). The nitrocellulose was blocked with 3% gelatin in TBS for at least 1 h at room temperature. Following blocking, the membranes were washed twice in TBS containing 0.5 mL/L 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 to creatine kinase (polyclonal to the MM isozyme of creatine kinase), glycogen phosphorylase (clone 10H5), glycogen debranching enzyme (clone 9063αDE), M-protein (clone A6), titin (clone T11), nebulin (clone NB1), and myosin heavy chain (clones F27 and F59). 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). The membranes were washed twice in TTBS for 5 min followed by a 10 min wash in TBS. Reactivity of the primary antibody with its antigen was detected by incubation of the blots with the alkaline phosphatase substrate solution consisting of 15 mg of BCIP and 30 mg of NBT dissolved separately in 1 mL of *N*,*N*-dimethylformamide and added to 100 mL of 0.1 M $NaHCO_3$ and 1.0 mM $MgCl_2$, pH 9.8.

Densitometry. SDS−PAGE proteins bands were scanned using an IS-1000 digital imaging system (Alpha Innotech Corp., San Leandro, CA). Densitometry was also used to determine the relative quantity of protein that reacted with the primary antibodies from the Western blot analyses and reported as arbitrary peak area values.

Statistical Analysis. Means for the densitometry values of each band in SDS−PAGE gels for the HIS extract of 32 carcass halves (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 half of eight carcasses (four RG and four NG) 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 combination of rate of post-mortem glycolysis and time of chilling.

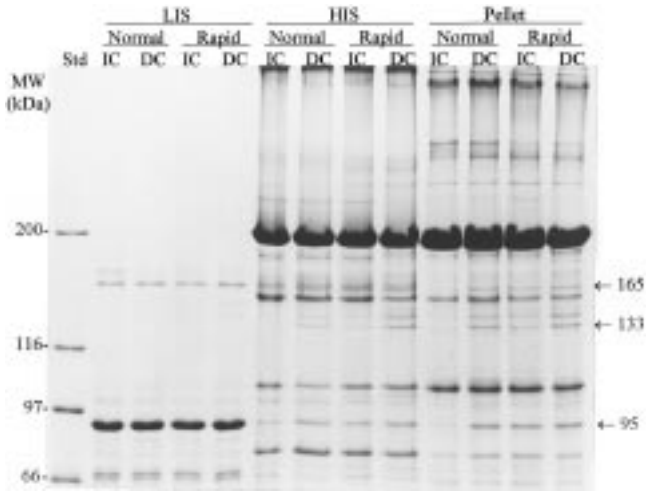

Figure 1. Coomassie brilliant blue stained 8% (100:1) gel of SDS−PAGE analysis of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC). Each lane contains 20 μg of protein from extraction with LIS buffer, HIS buffer, or proteins insoluble in HIS (pellet).

RESULTS AND DISCUSSION

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 regardless of the rate of post-mortem glycolysis or time of chilling. However, there were obvious differences in banding patterns due to the rate of post-mortem glycolysis and time of chilling for both proteins extracted in HIS buffer and pellet samples (Figure 1). 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.

Two bands corresponding to proteins of 95 and 165 kDa could be observed in both extracts as well as in the insoluble fraction at different intensities. 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 post-mortem glycolysis was rapid or when chilling was delayed. Another possibility is that these proteins are fragments of larger myofibrillar proteins with the same molecular weights as proteins in the LIS extract.

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

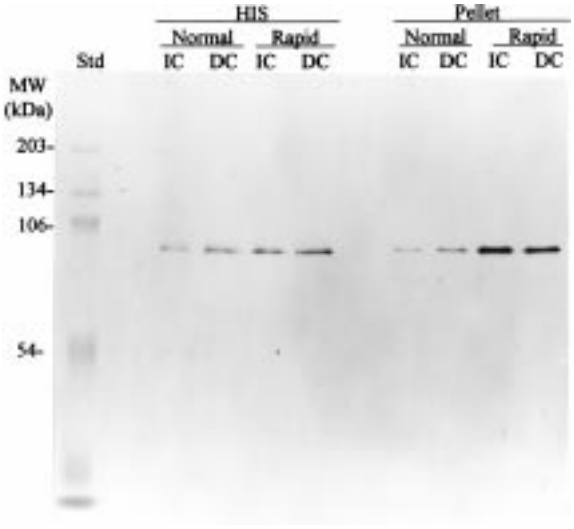

Figure 2. Western blot of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC) probed with anti-glycogen phosphorylase. Each lane contains 20 μg of protein from extraction with HIS buffer or proteins insoluble in HIS (pellet).

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.

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 2). Observation of Western blot results suggested that there were obvious 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 1). 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 LIS 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 LIS 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 in PSE pork (Offer, 1991) and RG turkey breast

Table 1. Arbitrary Peak Area Values from Densitometry of Western Blots for Proteins from NG and RG Turkey Breast and the Effect of Delaying the Chilling Procedure

	protein sample	glycogen phosphorylase	creatine kinase	M-protein
rate of post-mortem glycolysis				
NG[a]	HIS[c]	77.3	90.6	94.9
RG[b]	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[a]	pellet[d]	105.8	8.5	30.5
RG[b]	pellet	197.0	12.0	38.1
P value		0.02	0.14	0.07
SEM ($n = 4$)		30.5	2.5	3.8
start of immersion chilling				
20 min post-mortem	HIS	91.3	94.8	102.5
110 min post-mortem	HIS	108.6	106.0	98.3
P value		0.04	0.29	0.69
SEM ($n = 8$)		6.7	9.7	10.2
20 min post-mortem	pellet	124.8	10.4	31.0
110 min post-mortem	pellet	178.0	10.1	37.6
P value		0.14	0.91	0.01
SEM ($n = 8$)		31.3	2.1	1.6

[a] Normal glycolyzing (pH >6.00 at 15 min post-mortem). [b] Rapid glycolyzing (pH ≤5.80 at 15 min post-mortem). [c] HIS, high ionic strength extract (0.55 M KCl). [d] Pellet, proteins insoluble in HIS.

(Pietrzak et al., 1997) by LIS buffer. 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). We observed significantly greater amounts of creatine kinase 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 1.

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 results of our study indicate an increased association of these two proteins with myofibrillar proteins, but the effect on extractability of myofibrillar proteins is unclear.

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 (data not shown) indicating that, unlike glycogen phosphorylase, extraction of glycogen debranching enzyme in LIS was not affected by the rate of post-mortem glycolysis or time of chilling.

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 3 is a Western blot of HIS-extracted proteins

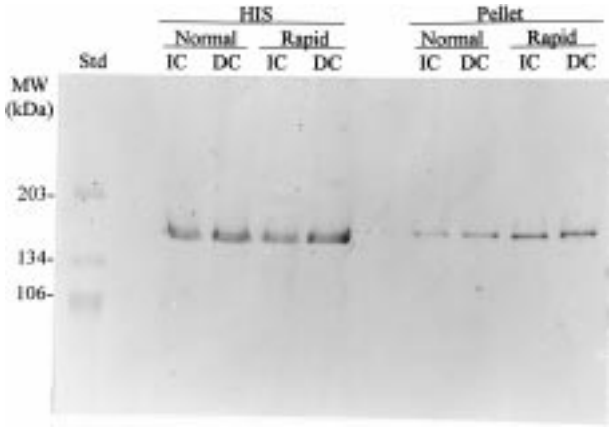

Figure 3. Western blot of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC) probed with anti-M-protein. Each lane contains 20 μg of protein from extraction with HIS buffer or proteins insoluble in HIS (pellet).

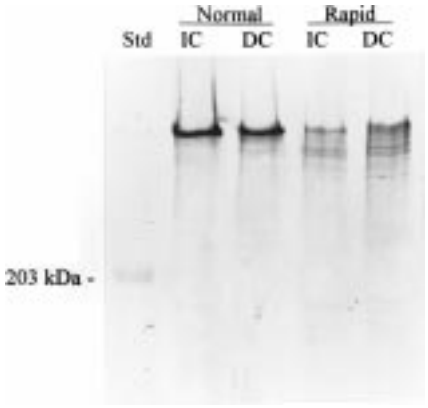

Figure 4. Western blot of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC) probed with anti-nebulin. Each lane contains 20 μg of protein from 24 h post-mortem myofibril preparations.

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 1). 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. 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.

Titin and Nebulin. Titin and nebulin are extremely large cytoskeletal proteins (approximately 3000 and 600−900 kDa, respectively; Huff-Lonergen et al., 1996). Degradation of either titin or nebulin could result in protein fragments of substantial size; however, the 165 kDa protein did not react with antibodies to either titin or nebulin. The antibody specific to titin reacted with the parent molecule and a very large degradation product known as T2 (Taylor et al., 1995) (data not shown). Detected degradation products of nebulin were also very large (Figure 4). Titin or nebulin degradation as a source for smaller polypeptides cannot be ruled out, because fragments may not contain the epitopes T11 and NB1 recognize. Indeed, Ho et al. (1996) were able to identify numerous 39−130 kDa degradation products of titin using a monoclonal antibody different from that used in our study.

Table 2. Arbitrary Peak Area Values from Densitometry of Western Blots for Nebulin from NG and RG Turkey Breast and the Effect of Delaying the Chilling Procedure

	nebulin in myofibril preparations
rate of post-mortem glycolysis	
NG[a]	376
RG[b]	234
P value	0.03
SEM ($n = 4$)	60
start of immersion chilling	
20 min post-mortem	303
110 min post-mortem	308
P value	0.93
SEM ($n = 8$)	43

[a] Normal glycolyzing (pH >6.00 at 15 min post-mortem). [b] Rapid glycolyzing (pH ≤5.80 at 15 min post-mortem).

In the present study, post-mortem degradation of titin to its large fragment T2 was not affected by the rate of post-mortem glycolysis or by the time chilling was initiated. Our results do not support the reports of Pospeich 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 3 h post-mortem (data not shown). However, NB1 revealed increased degradation of nebulin in RG samples compared to NG samples by 24 h post-mortem (Figure 4; Table 2). Delayed chilling did not affect the degradation of nebulin. Therefore, the effect of nebulin degradation on protein extractability is unclear, because degradation appears to be independent of changes to myofibrillar protein extractability. On average, densitometry results indicated 58% of nebulin in RG samples was degraded compared to 33% in NG controls. Other investigators have reported increased degradation of nebulin in RG turkey breast (Pospiech et al.,1992) and 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 our results and 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 our study were selected with similar ultimate pH values to observe the effect of the rate of pH decline post-mortem on individual muscle proteins without the influence of different ultimate pH values.

Myosin Heavy Chain (MHC). 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 5A). Interestingly, one of the MHC fragments was the same molecular weight as the band that reacted with

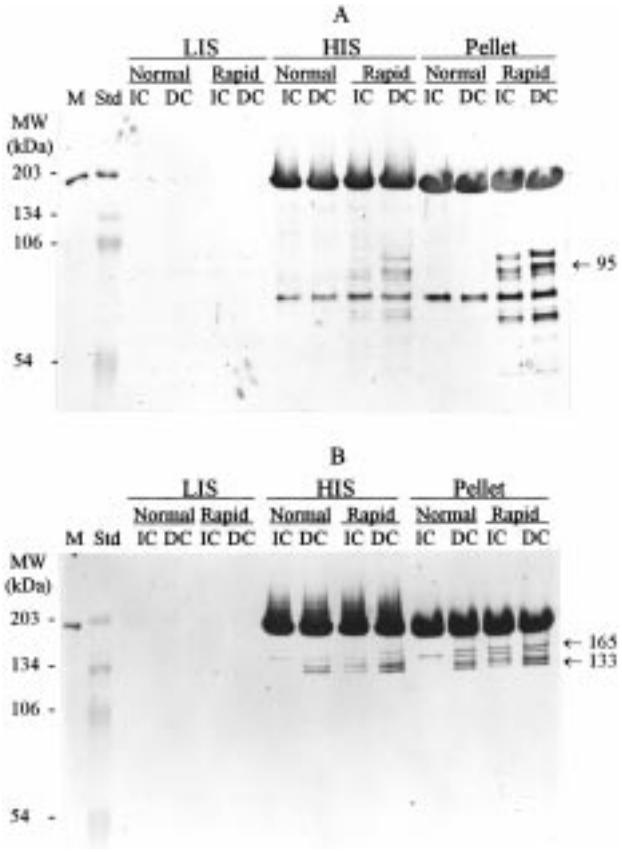

Figure 5. Western blots of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC) probed with (A) anti-heavy meromyosin (clone F59) or (B) anti-light meromyosin (clone F27). Each lane contains 20 μg of protein from extraction with LIS buffer, HIS buffer, or proteins insoluble in HIS (pellet).

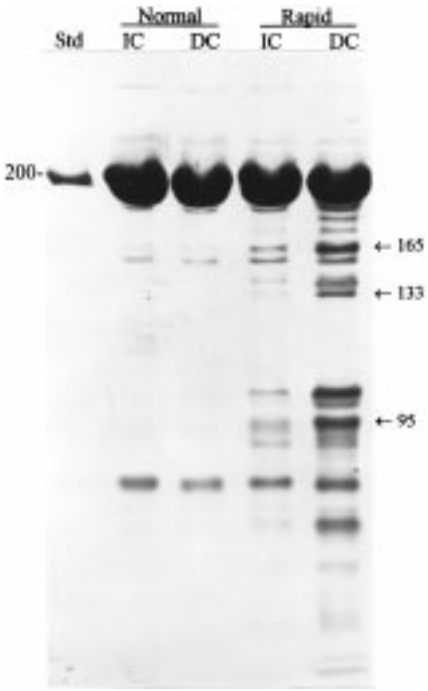

Figure 6. Western blot of turkey breast muscle proteins from NG and RG turkey carcass halves chilled immediately (IC) or delay chilled (DC) probed with both anti-heavy meromyosin (clone F59) and anti-light meromyosin (clone F27). Each lane contains 20 μg of protein from 24 h post-mortem myofibril preparations.

anti-glycogen phosphorylase (95 kDa). 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 5B). 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 5A); however, strong reactions for samples from other NG/DC carcass halves occurred (Figure 5B). 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.

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 reduced MHC (200 kDa) in DC samples. This 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 suggested 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, in our results, 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 6 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 15−20% 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 myofibrillar protein extractability.

O'Halloran et al. (1997) reported that both μ-calpain activity and release of cathepsins B and L are increased in RG beef compared to normal controls. Calpains have been implicated in the post-mortem degradation of titin, nebulin, and Z-line proteins (Taylor et al., 1995). However, post-mortem degradation of myosin is usually attributed to cathepsin activity (Zeece and Katoh, 1989; Whipple and Koohmaraie, 1991), which is optimum at temperatures near 37 °C and low pH (4.0−6.0) values (Greaser, 1986). In our study, myosin degradation was evident in samples collected 3 h post-mortem and increased by delay chilling when carcass temperatures would be elevated. However, nebulin degradation was minimal in 3 h post-mortem 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 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 chicken P. major homogenates adjusted to pH 5.5 and heated to 40 °C for 6−24 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 post-mortem. 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.

Implications. Our results indicate that there are changes involving several proteins in RG and DC turkey breast meat. These changes include a reduction in the extractability of at least two sarcoplasmic proteins and alterations in M-protein extractability. Perhaps the most notable change was the increased degradation of both nebulin and myosin in the breast meat of RG carcasses. This is the first study associating rapid post-mortem glycolysis or delayed chilling with degradation of turkey breast myosin. Additionally, the absence of a significant interaction between the rate of post-mortem glycolysis and delay chilling suggested increased MHC degradation was additive for RG/DC turkey breast samples. Delay chilling of RG turkey breast may become a useful tool leading to new discoveries because subtle differences may be exaggerated and become easier to detect due to this additive affect. Conversely, and perhaps more importantly to poultry processors, this work demonstrated that rapid chilling minimized myosin degradation.

Because myosin fragments were observed primarily in myofibrillar protein samples from RG and DC carcasses that also had lower protein extractability, myosin degradation appears to be detrimental to the protein extractability of turkey breast muscle. This may have several consequences as myofibrillar protein extractability is positively correlated to cook yield and cooked meat gel strength (Rathgeber et al., 1999). Wang and Xiong (1998) also observed that when bovine cardiac myosin was degraded by cathepsins, the strength of the resulting cooked meat gels was decreased. The influence of post-mortem degradation of myosin and other proteins on turkey meat quality is an area of research that warrants further exploration as proteolysis of muscle proteins could be responsible for costly losses in the meat industry.

ABBREVIATIONS USED

DC, delay chilled; HIS, high ionic strength; IC, immediately chilled; LIS, low ionic strength; MHC, myosin heavy chain; NG, normal glycolyzing; PSE, pale soft exudative; RG, rapid glycolyzing; SDS−PAGE, sodium dodecyl sulfate polyacrylamide electrophoresis; SSS, standard salt solution; TBS, Tris-buffered saline; TTBS, Tris-buffered saline with Tween 20.

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