Thermally Induced Changes in Protein Extractability of Postrigor Turkey Breast Meat Measured by Two Methods

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ABSTRACT
Extractability of sarcoplasmic and myofibrillar proteins was reduced in postrigor turkey breast meat held at 40 °C for as short as 30 min. These reductions in protein extractability were successfully detected, using either a filtration-based methodology or one based on centrifugation. However, the coefficient of variation for the filtration method was as much as 4 times greater than for centrifugation. Additionally, the filtration method overestimated sarcoplasmic protein extractability due to the inability to exclude myofibrillar proteins. Centrifugation results indicated the extractability of proteins in 0.55 M KCl was reduced to 52% of controls for samples held at 40 °C for 120 min. Additionally, holding postrigor turkey breast muscle at 40 °C resulted in increased myosin degradation.

Key Words: turkey, protein extractability, postrigor heating, protein degradation

INTRODUCTION
When evaluating the quality of meat, investigators often use a measure of protein extractability, which affects yield, texture, gel strength, and palatability of processed meat products (Camou and Sebranek, 1991; Rathgeber et al., 1999a). Low protein extractability is typical of pork meat commonly described as pale, soft, and exudative (PSE) (Boles et al., 1992; van Laack et al., 1993). Typically, PSE meat results from the combination of extremely low muscle pH and high carcass temperature early postmortem (Offer, 1991). Unusually rapid postmortem glycolysis induced by antemortem stress is often the reason for low muscle pH early postmortem.

Because changes in PSE meat are a result of low muscle pH in combination with elevated carcass temperatures, some investigators have taken postrigor porcine muscle of low pH and returned it to physiological temperatures in an attempt to create PSE meat (Fischer et al., 1979; Sakata et al., 1981, 1983). Postrigor heated meat was referred to as PSE-like due to some similarities to PSE meat. Fischer et al. (1979) held postrigor pork at 40 °C for 90 min and reported a reduction in solubility and activity of the enzyme glycogen phosphorylase. Sakata et al. (1983) also held postrigor pork at 40 °C for 90 min and found the extractability of sarcoplasmic proteins was 70% of the unheated controls.

There are a number of reports that a meat quality problem similar to PSE pork has been identified in turkey breast meat (Barbut 1993; Hollingsworth, 1993; Ferket, 1995; Pietrzak et al., 1997; Rathgeber et al., 1999a, 1999b). It appears that the combination of elevated carcass temperature and low muscle pH play a major role in meat quality problems with turkey breast (Hollingsworth, 1993; Ferket, 1995; Pietrzak et al., 1997; Rathgeber et al., 1999a). However, it has not been established whether PSE-like characteristics occur when turkey breast meat is heated postrigor. Therefore, one objective of this study was to determine whether holding postrigor turkey P. major at 40 °C affects protein extractability.

Choosing a method for measuring protein extractability requires consideration of a number of variables. 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 a combination of both are the most commonly used systems for separation of extractable proteins from the remaining proteins (Patel and Fry, 1987). There are advantages and disadvantages for each method of protein separation and the use of either may depend largely upon what equipment is available to the investigator.

Fischer et al. (1979) and Sakata et al. (1983) evaluated changes in extractability of sarcoplasmic proteins in PSE-like pork using centrifugation-based methods. Boles et al. (1992) reported the extractability of both sarcoplasmic and myofibrillar proteins was reduced for genetically stress-sensitive pork. These researchers used a method based on centrifugation of the samples. Total protein extractability in a high-ionic-strength buffer was reduced in PSE pork samples in a study by van Laack et al. (1993), who used a filtration-based method. In our study, protein extractability of postrigor heated turkey breast meat was measured using both a filtration-based method and a centrifugation-based method. The suitability of each method for measuring both sarcoplasmic and myofibrillar protein extractability was determined.

In addition to measuring extractability of groups of muscle proteins, the extractability of individual proteins was investigated through the use of SDS-PAGE. Changes to the extractability of the heavy chain subunit of myosin, specifically, were monitored with the use of Western blotting.

MATERIALS & METHODS
Sample preparation
Tom turkeys were raised to 116 d of age and processed at a commercial slaughter plant. Five carcasses were chosen at random following immersion-chilling and stored at 4 °C until 48 h postmortem when the P. major was removed, cut into about 100-g samples, vacuum-packaged, and frozen at −40 °C. Samples were removed from −40 °C storage and tempered in a −1 °C cooler for 12 h prior to the experiment. The fat and connective tissue were trimmed from tempered samples. These samples were ground through a 20-mm plate followed by grinding through a 3-mm plate using chilled equipment.

For each turkey breast, a 40-g sample of ground meat was homogenized (Polytron PT 10/35, Brinkman Instruments, Mississauga, Ont., Canada) in 200 mL water (4 °C) containing 1 mM sodium azide as an antimicrobial agent (Yamamoto et al., 1979) and

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2 mM EDTA to inhibit calpain activity (Uytterhaegen et al., 1994). Each meat mixture was homogenized for 6 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, Ont., Canada). Kjeldahl nitrogen (AOAC, 1990) of each homogenate was determined and multiplied by 6.25 to obtain a measure of total protein of the samples. Six-gram (± 0.03 g) samples of homogenate were removed from this pool and added to 50 mL centrifuge tubes, capped to minimize evaporation, and put on ice. For each turkey breast 32 of these homogenate samples were assigned at random to one of 16 possible treatments, with 2 samples of homogenate were removed from this pool and added to 50 mL water bath for 30 min. After heat treatment, the samples were homogenized for 10 s and placed in an ice water bath for 15 min.

**Protein extractability calculations**

The protein extractability was determined by multiplying the number of milligrams of protein per milliliter by the original volume of the extraction solutions. 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 extracted.

**Heat treatment**

Samples were placed in an ice water bath or 40 °C covered water bath for 30 or 120 min. After heat treatment, the samples were placed in ice water for 15 min.

**Protein extraction**

To each homogenate sample 20 mL chilled buffer (4 °C) was added. A low-ion-strength phosphate buffer (LIS; 0.06 M potassium phosphate buffer, 1 mM NaNO₃, 2 mM EDTA, pH 7.3) was added to extract the sarcoplasmic proteins, or a high-ion-strength buffer (HIS; 0.06 M potassium phosphate buffer, 0.69 M KCl, 1 mM NaNO₃, 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 water added to each gram of meat taken into account (from preparation step), the extraction solutions had similar ionic strength to commonly used buffers; 0.05 M potassium phosphate buffer, 1 mM NaNO₃, 2 mM EDTA, pH 7.3 (Boles et al., 1992) and 0.05 M potassium phosphate buffer, 0.55 M KCl, 1 mM NaNO₃, 2 mM EDTA, pH 7.3 (Boles et al., 1992; van Laack et al., 1993; Xiong et al., 1993). Following the addition of extraction buffer the samples were homogenized for 10 s and placed in an ice water bath for 30 min.

**Separation methods**

**Filtration.** 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, we placed a 40-μm-pore-size filter (VWR Scientific, Mississauga, Ont., Canada) on top of a Whatman No. 1 filter paper. Filtration was facilitated by a vacuum generated by a water aspiration unit. The protein in the filtrate was determined using the buret procedure at 540 nm (Gornall et al., 1949). Bovine serum albumin was used as the protein standard (van Laack et al., 1993). Protein measurements were made within 30 min of filtration and again after 2 d of refrigerated storage (4 °C) to determine whether proteins extracted remained in solution.

**Centrifugation.** The samples assigned to the centrifugation treatment were centrifuged at 17,500 × 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.

**Protein extractability calculations**

The protein extractability was determined by multiplying the number of milligrams of protein per milliliter by the original volume of the extraction solutions. 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 extracted.

**Electrophoresis**

Sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) was performed as outlined by Laemmli (1970), using a Mini-Protein® II electrophoresis unit (BioRad Laboratories, Hercules, Calif., U.S.A.). The protein samples extracted in LIS and HIS for each treatment combination were run on 11% (100:1 acrylamide:N,N′-bis-methylene acrylamide) resolving gels with a 4% (37.5:1) stacking gel. Gels were run at 35 mA, constant current, until the dye front reached the bottom. Gels were stained 30 min in 0.1% Coomassie brilliant blue R-250, 10% glacial acetic acid, 40% methanol and destained twice in the same solution omitting the Coomassie blue. Gels used for Western blot analysis were equilibrated in transfer buffer for 15 min prior to transfer.

**Immunoblotting**

Proteins run on SDS-PAGE gels were transferred to nitrocellulose membranes (0.45 μm, BioRad Laboratories, Hercules, Calif., U.S.A.) at 40 volts for 2.5 h, using a Transblot Cell® (BioRad Laboratories, Hercules, Calif., U.S.A.). The transfer buffer was 25 mM Tris, 192 mM glycine, 0.1% SDS with 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 in TBS for at least 1 h at room temperature. Following blocking, the membranes were washed twice in TBS containing 0.5% gelatin in TBS containing a goat anti-mouse secondary antibody conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo., U.S.A.) for at least 1 h at room temperature. Membranes were incubated with the primary antibody in 1% gelatin in TBS overnight at room temperature. Membranes were washed in Tris-buffered saline for at least 1 h at room temperature. Membranes were washed twice in TBS for 5 min followed by a 10-min wash in TBS. At this point, 15 μg of 5-bromo-4-chloro-3-indolyl phosphate and 30 μg of nitro blue tetrazolium were dissolved in 1 mL N,N-dimethylforamide each and added to 100 mL of 0.1 M NaHCO₃, 1.0 mM MgCl₂ (pH 9.8). The transfers were incubated in this mixture to initiate color development, which was stopped by washing in deionized water.

**Statistical analysis**

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

**RESULTS & DISCUSSION**

The results of protein extractability measurements for postrigor turkey breast meat held at 0 °C (controls) and 40 °C are presented in Fig. 1. 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 the heating of postrigor turkey breast meat. Reductions in protein extractability due to postrigor heating were detected when either the filtration method or the centrifugation method was used. When the protein extractability measurements determined by filtration were analyzed separately from the centrifugation measurements, it was apparent that 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 2.5 times that of the centrifugation method (Table 1). The coefficient of variation for sarcoplasmic protein extraction was 4 times larger for filtration compared to centrifugation.

Part of the reason for the increased variation in protein extractability measurements when filtration was used can be explained by the use of Western blot analysis of extracted proteins. Initially, SDS-PAGE analysis of turkey breast proteins extracted with the LIS revealed different banding patterns for samples subjected to filtration compared to centrifugation. In particular, a 200-kDa protein present in the filtered samples was absent in the samples centrifuged. Fig. 2 is a Western blot of these proteins labeled with anti-myosin heavy chain antibodies that illustrates a significant amount of myosin heavy chain (MHC) in the LIS filtrate. MHC is not normally soluble in LIS (Pearson and Young, 1989). 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, MHC was absent in LIS samples following centrifugation. Other evidence for the presence of insoluble proteins in the filtered LIS samples was apparent when protein extractability measurements were made for the second time at 48 h after filtration. At this time there was an obvious precipitate in the filtered LIS samples that was absent in the centrifuged samples. Protein extractability measurements for filtered samples extracted with either LIS and HIS buffer made after 48 h of refrigerated storage were significantly (P < 0.05) lower than measurements made within 30 min of the filtration procedure (Fig. 3). Extractability of centrifuged proteins was the same after refrigerated storage as values taken initially indicating minimal precipitation of proteins. Western blot analysis of the precipitate from filtered samples extracted in LIS buffer indicated that MHC was a
substantial component of this fraction (Fig. 4).

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. Those authors reported significantly higher solubility results for filtration of caseinate and whey protein isolate using Whatman No. 1 filter paper compared to centrifuged samples. In our study with postmortem 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. In our 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 impractical rate.

Because filtration failed to separate extractable proteins from the remainder of the proteins as successfully as the centrifugation method, data from the centrifugation method are more reliable. Therefore, for the remainder of the results and discussion, only centrifugation results will be presented. For 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. These results were similar to the findings of Sakata et al. (1983) who reported extractability of sarcoplasmic proteins for postmortem porcine meat held at 40 °C for 90 min was reduced by 30%. In comparison, Lopez-Bote et al. (1989) reported sarcoplasmic protein extractability from turkey breast meat was 7% lower for birds that exhibited a rapid rate of postmortem glycolysis.

Fischer et al. (1979) and Sakata et al. (1981, 1983) held postmortem porcine muscle at 40 °C as a model to produce PSE-like meat; however, 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 with regard to protein functionality than sarcoplasmic proteins. In our study, when postmortem turkey breast muscle samples were heated to 40 °C for 30 min, protein extracted with HIS was 70% 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.

It may also be useful to quantify changes in myofibrillar protein extractability compared to sarcoplasmic protein extractability. Lopez-Bote et al. (1989) reported that myofibrillar protein extractability for PSE porcine meat was 59% of that extracted from normal meat. They determined myofibrillar protein extractability by subtracting the amount of protein extracted in LIS from the total protein extracted with HIS. If we use this calculation, the myofibrillar proteins extracted were 54% and 36% of the controls after 30 and 120 min at 40 °C, respectively. Rathgeber et al. (1999a) compared protein extractability of turkey breast meat with a pH ≤ 5.8 at 15 min postmortem to controls with a pH of > 6.0 at 15 min postmortem. Myofibrillar protein was determined by first removing sarcoplasmic proteins from the samples. The myofibrillar protein extractability of the breast meat with low pH early postmortem was 66% of the controls. These results indicate that the extractability of myofibrillar proteins is more sensitive to conditions of low muscle pH and elevated temperatures than the extractability of 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.

SDS-PAGE analysis was employed to investigate individual breast muscle proteins involved in reduced protein extractability due to postmortem heating. The intensity of the 200-kDa band for SDS-PAGE of the proteins extracted in HIS buffer was reduced for samples from meat exposed to 40 °C for 120 min compared to controls held at 0 °C. This suggested that less myosin was extracted upon exposure to 40 °C postmortem. 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 (Fig. 5). Fragments of MHC of 152, 142, 133, 110, 95, 93, 84, 73, and 71 kDa would account for the reduction in the intensity of the 200-kDa band on SDS-PAGE. Only one fragment of MHC (about 110 kDa) was present in the LIS supernatant of samples, and this was for samples held at 40 °C for 120 min (Fig. 3). The 110-kDa fragment cross-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 regarding the solubility of heavy meromyosin. Intact MHC is insoluble in LIS buffer; however, heavy meromyosin is soluble at
lower ionic strength (Lowey, 1971; Pearson and Young, 1989). Rathgeber et al. (1999b) reported increased degradation of MHC in turkey breast meat samples from carcasses with low muscle pH early postmortem compared to normal controls. However, the banding pattern of the degradation products of MHC were slightly different than shown here for the postmortem heated samples. Additionally, Rathgeber et al. (1999b) did not find heavy meromyosin in the LIS extraction solutions of turkey breast samples.

The increased degradation of myosin heavy chain in the postmortem heated turkey breast samples may significantly alter the extractability of myosin and other proteins of the myofibril. The differences in MHC degradation in this study compared to Rathgeber et al. (1999b) may be due to the extent of MHC degradation in postmortem heated samples compared to samples with low early postmortem muscle pH. The differences may also be due to differences in the activity of endogenous proteases. The suitability of postmortem heating as a model for rapid postmortem glycolysis may not be appropriate. However, postmortem heating could be an acceptable model for postmortem temperature abuse of meat.

CONCLUSIONS

The CENTRIFUGATION METHOD FOR MEASURING PROTEIN EXTRACTABILITY was more appropriate for measuring differences in protein extractability for turkey breast samples heated postmortem than the filtration-based method. The failure of the filtration-based method to effectively remove insoluble protein led to increased variability compared to the centrifugation-based method. This was especially evident when low-ionic-strength buffer was used to extract the sarcoplasmic proteins as indicated by the presence of MHC in the western blots of these samples. The reduction of protein extractability for postmortem heated samples was of a similar magnitude to previous reports for turkey breast proceeding through rigor at an accelerated rate; however, differences in the banding patterns for the fragments of MHC degradation suggests that different mechanisms may be involved. The occurrence of extensive degradation of MHC for turkey breast heated postrigor was of a similar magnitude to previous reports for turkey breast proceeding through rigor at an accelerated rate; however, differences in the banding patterns for the fragments of MHC degradation suggests that different mechanisms may be involved. The occurrence of extensive degradation of MHC for turkey breast heated postrigor warrants further investigations into the relationship between proteolysis of myofibrillar proteins and protein functionality.

REFERENCES


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