Effect of pH and postmortem aging on protein extraction from broiler breast muscle

M. Eady, D. Samuel, and B. Bowker

USDA, Agricultural Research Service, Richard B. Russell Research Center, Athens, GA 30605

ABSTRACT This study determined the effects of extraction buffer pH and postmortem aging on the extraction of salt-soluble and water-soluble proteins from broiler pectoralis muscle. Deboned broiler breast fillets were collected at 4 h postmortem, packaged, and then stored at 4°C until 1, 5, or 8 d postmortem. After the designated aging period, salt-soluble and water-soluble protein extractions were performed using buffers at 7 different pH levels (pH 5.4, 6.4, 6.9, 7.2, 7.5, 8.0, 9.0). Protein concentrations of the extracts were measured and SDS-PAGE analysis was performed. Salt-soluble protein concentration increased ($P < 0.0001$) as buffer pH increased from pH 5.4 to 6.9 and then remained unchanged from pH 6.9 to 9.0. Water-soluble protein concentration increased ($P < 0.0001$) as buffer pH increased from pH 5.4 to 7.2 and then remained unchanged from pH 7.2 to 9.0. There was not a significant extraction buffer pH by aging treatment interaction for the total protein concentration of either the salt-soluble or water-soluble protein extracts. The protein concentrations of salt-soluble extracts were similar at both 1 and 8 d postmortem but lower ($P < 0.0001$) at 5 d postmortem. The protein concentrations of water-soluble extracts were similar at both 1 and 5 d postmortem, but higher ($P < 0.0001$) at 8 d. Both extraction buffer pH and postmortem aging influenced the SDS-PAGE protein profiles of salt-soluble and water-soluble protein extracts from breast muscles. Data demonstrate that postmortem aging and extraction buffer pH influence both the total amount and the composition of the myofibrillar and sarcoplasmic proteins that can be extracted from broiler breast fillets.

Key words: aging, broiler breast meat, myofibrillar protein, pH, sarcoplasmic protein

INTRODUCTION

Meat quality in both fresh and processed poultry products can be influenced by muscle protein functionality, which can be defined as any inherent or process-generated protein characteristic that affects physical and sensory attributes of raw and finished products (Xiong and Kenney, 1999). Protein solubility, or extractability, is a functional property used to classify muscle proteins and a physicochemical trait often used as an indicator or precursor for other functional properties. Extractability of muscle proteins influences water-binding, emulsion capacity and stability, adhesion of meat pieces, gelation, and textural characteristics of meat products. Before strategies can be developed to optimize functional properties and meat quality traits that depend on protein extractability in chicken products, fundamental research is needed to understand factors that influence protein extractability in fresh chicken meat.

The extraction of proteins from meat is a complex phenomenon that is influenced by not only the parameters of the extraction process itself but also by the diversity of the proteins present, the intricately organized structure of the tissue, and the postmortem changes that occur during the transformation of muscle to meat. In laboratory settings, extraction conditions such as buffer pH, ionic strength, type of salt, extraction volume, and homogenization influence muscle protein extractability (Regenstein and Rank Stamm, 1980; Richardson and Jones, 1987; Lan et al., 1993; Munasinghe and Sakai, 2004; Thomas et al., 2007). Several studies have demonstrated that the extraction of salt-soluble proteins from isolated myofibrils and muscle homogenates prepared from chicken is influenced by extraction buffer pH 5.8 to 6.6 (Xiong and Brekke, 1991; Xiong and Blanchard, 1994; Lesiow and Xiong, 2003). However, the effects of extracting chicken muscle proteins with buffers at pH values greater than 7.0 are relatively unknown. In regards to intrinsic muscle characteristics in chicken, it
has been demonstrated that protein extractability is influenced by muscle type (Amato et al., 1989; Xiong and Blanchard, 1994) and rigor state (Xiong and Brekke, 1991). Several studies have also shown that the extractability of chicken muscle proteins in slightly acidic to neutral buffers (pH 6.0 to 7.0) is also influenced by the duration of the postmortem storage period prior to extraction (Sayre, 1968; Regenstein and Rank Stamm, 1980; Li-Chan et al., 1986; Xiong and Brekke, 1989). It is not known, however, if the pH sensitivity of muscle protein extraction over a wide range of pH values (5.4 to 9.0) is influenced by extended postmortem aging (>3 d) of chicken meat.

Due to their demonstrated importance in processed meats, studies on muscle protein extractability have focused primarily on myofibrillar proteins and less on the water-soluble sarcoplasmic proteins. Past data, however, have shown that the denaturation of sarcoplasmic proteins can influence the extractability of myofibrillar proteins (Scopes, 1964). Although it is well known that myofibrillar protein denaturation influences water-holding capacity in pork (Warner et al., 1997), it has been shown that myofibrillar proteins from broiler breast muscles are resistant to denaturation (Van Laack and Lane, 2000). Although it was not thought to be a causal relationship, Van Laack et al. (2000) observed a strong positive correlation (r = 0.66) between sarcoplasmic protein solubility and water-holding capacity in broiler breast muscles, but found that myofibrillar protein solubility and water-holding capacity were unrelated. Additionally, Xiong and Anglemier (1989) observed that the water-soluble fraction of muscle contained proteins thought to be of myofibrillar origin as a result of proteolysis during postmortem aging. Collectively, these data suggest that to fully understand muscle protein extractability and functionality, both the myofibrillar and sarcoplasmic protein fractions of muscle should be considered.

Thus, the objective of this study was to determine the combined effects of extraction buffer pH and postmortem aging on the extractability of both salt-soluble and water-soluble proteins in broiler breast fillets. Total protein concentration of the extracts was used as an indicator of overall protein extractability. It was hypothesized that extraction buffer pH and muscle aging would not only affect overall protein extractability but would also influence the protein profiles of salt-soluble and water-soluble protein extracts from chicken muscle. To test this hypothesis, SDS-PAGE analysis was used to characterize the specific protein compositions of the muscle extracts.

MATERIALS AND METHODS

Sample Collection and Meat

Quality Measurements

Deboned breast fillets (n = 12) were collected from a single deboning line in a commercial poultry processing facility at 4 h postmortem. Breast fillets were from 6-wk-old broilers of similar genetic background and rearing conditions. Samples were selected from the production line based on a visual color assessment to avoid fillets considered pale, soft, and exudative or dark, firm, and dry. Fillets were stored in a sealed bag at 4°C overnight. At 24 h postmortem, lightness values (L*) were recorded on the dorsal surface of each fillet using a Konica Minolta 400 colorimeter (Tokyo, Japan) and muscle pH values were determined with an Oakton pH meter equipped with a Cole-Palmer glass spear-tip probe (Vernon Hills, IL). Excess connective tissue and fat were removed and initial fillet weights were recorded. Samples were randomly assigned to 1 of 3 aging treatments (1, 5, or 8 d postmortem), individually vacuum packaged, and stored at 4°C until the day of analysis. The entire experiment was replicated 3 times for a total of 36 breast fillets.

Protein Extractions

From each breast fillet, water-soluble and salt-soluble proteins were extracted at 7 pH levels (pH 5.4, 6.4, 6.9, 7.2, 7.5, 8.0, and 9.0) according to the modified procedure of Barbut et al. (2005). The medial portion of each pectoralis major muscle was removed and trimmed of fat and connective tissue. Approximately 100 g of each muscle sample was minced for approximately 12 s in a Rival 1.5C food processor (Boca Raton, FL). Two grams of minced sample was then added to 20 mL of cold sarcoplasmic extraction buffer (0.025 M Na₂HPO₄, adjusted to appropriate pH). Samples were then homogenized for 60 s at 35,000 rpm using an Omni THQ digital homogenizer (Kennesaw, GA) and centrifuged at 7,000 × g for 15 min at 4°C. The supernatant was decanted and saved as the water-soluble protein extract. Then 20 mL of cold myofibrillar extraction buffer (0.025 M Na₂HPO₄, 0.6 M NaCl, adjusted to appropriate pH) was added to the pellet, and samples were rehomogenized at the previous settings and stored overnight at 4°C. Samples were then centrifuged at 7,000 × g for 15 min at 4°C and the supernatant was decanted and saved as the salt-soluble protein extract. Both the water-soluble and salt-soluble extractions were performed in duplicate. Protein extracts were diluted 1:50 in their respective buffers and protein concentrations were measured in triplicate using the Coomassie Plus-The Better Bradford Assay Kit (Pierce, IL) with BSA standards and a Bio-Rad 550 (Hercules, CA) microplate reader.

SDS-PAGE

Protein extracts were diluted to 2 mg/mL in sample buffer [8 M urea, 2 M thiourea, 3% SDS (wt/vol), 75 mM dithiothreitol, 25 mM Tris-HCl (pH 6.8), 0.004% bromophenol blue] and denatured for 3 min in boiling water. Denatured samples were loaded (10 µg of protein/lane) onto precast 12% Tris-glycine polyacryl-
amide resolving gels. Gels were run on a Novex Midi Gel System (Life Technologies Corp., Carlsbad, CA) at 4°C using a constant 200 V in reservoir buffer described by Læmmli (1970). Gels were stained in a solution of 0.1% (wt/vol) Coomassie brilliant blue R-250, 40% methanol, and 7% acetic acid for 2 h and destained in a solution of 40% methanol and 7% acetic acid. Images were captured using a FluorChem M imaging system (ProteinSimple Inc., Santa Clara, CA) and AlphaView software (v 3.4, ProteinSimple Inc.) was used to quantify protein band densities. To account for slight gel loading variations, the intensity of each band was determined relative to the sum of the intensities of all bands within the gel lane. For each individual protein band, the relative intensity was then multiplied by the concentration of the protein extract to determine the relative amount of extractable protein.

**Statistical Analysis**

Data were analyzed using the GLM procedure in SAS software (Cary, NC). The model included extraction buffer pH (5.4, 6.4, 6.9, 7.2, 7.5, 8.0, and 9.0), post-mortem aging times (1, 5, and 8 d), and the extraction buffer pH × aging time interaction. Replication effects were analyzed, found to be insignificant, and not included in the final model. Means were separated using the least significant difference test at significance level $P < 0.05$.

**RESULTS AND DISCUSSION**

It is well-established that muscle protein solubility is related to meat quality and is influenced by the degree of denaturation that occurs during the transformation of muscle to meat early postmortem. In the current study, fillets were selected based on visual color, which was then confirmed by instrumental lightness ($L^*$) values and pH measurements at 24 h postmortem (Table 1) to avoid meat quality extremes. The average measurements observed in this study were very similar to the average measurements ($L^* = 62.1$ and pH = 5.96) of breast fillets that were classified as normal quality in a previous study utilizing samples from this particular processing plant (Qiao et al., 2002). The $L^*$ values observed in the current study were also in line with normal quality breast fillets in several studies reviewed by Smith and Northcutt (2009). Based on these comparisons, the $L^*$ and pH data of the raw fillets in this study were considered to be within the range of normal quality. Thus, it was determined that the differences in protein extractability observed in this study were due to the controlled factors of extraction buffer pH and postmortem aging rather than inherent differences in initial fillet quality.

The effects of extraction buffer pH on the protein concentrations of muscle extracts are shown in Figure 1. Protein concentrations of the salt-soluble extracts increased ($P < 0.0001$) with the pH of the buffer increased from pH 5.4 to 6.9 and then remained unchanged from pH 6.9 to 9.0. Similarly, protein concentrations of the water-soluble extracts increased ($P < 0.0001$) as the pH of the buffer increased from pH 5.4 to 7.2 and then remained unchanged from pH 7.2 to 9.0. Both the salt-soluble and water-soluble protein extracts exhibited the lowest protein concentration at pH 5.4. Protein concentrations of the water-soluble extracts at pH 5.4 were 73% of maximum protein concentration at pH 7.5, whereas the protein concentrations of the salt-soluble extracts at pH 5.4 were only 48% of maximum. Thus, myofibrillar protein extractability seemed to be more pH sensitive than sarcoplasmic protein extractability.

In this basic study, the influence of pH on extract protein concentration suggests that buffer pH altered the surface properties of proteins and the protein to protein interactions sufficiently to affect overall extractability. The pH sensitivity of the salt-soluble extraction was likely a function of myosin. Because myosin is the primary muscle protein responsible for water-binding in meat, the observed pH effect on protein extractability in the current study closely parallels the effects of meat pH on water-holding capacity due to the net charge effect (Huff-Lonergan and Lonergan, 2005). As the pH of the extraction buffer approached the isoelectric point of myosin (pI = 5.4), the overall number of reactive groups on the muscle proteins for binding water diminished. The diminished capacity for the proteins to bind water likely resulted in the reduced protein solubility and extractability observed at pH 5.4. The reduced protein extractability at pH 5.4 may have also been partially due to steric effects within the myofibrils. At pH 5.4, the electrostatic repulsion between the myofilaments of the myofibrils would have been reduced, allowing the structures to pack more closely together, resulting in less open areas within the protein matrix for water to bind, which would have decreased protein extractability. In regards to the salt-soluble protein extracts, the presence of sodium chloride in the extraction buffer likely influenced the pH dependent response in protein extractability. The reduced extractability of water-soluble proteins at pH 5.4 was likely due to sarcoplasmic protein denaturation. It is also possible that the decrease in the net charge of both the salt-soluble and water-soluble proteins caused aggregation and the formation of a hydration layer around the proteins, which acted as a barrier to solubilization and extraction (Zhang et al., 2007).

These results are consistent with past data showing that extractability of myofibrillar proteins is dependent

<table>
<thead>
<tr>
<th>Trait</th>
<th>Fillet wt (g)</th>
<th>pH&lt;sub&gt;24h&lt;/sub&gt;</th>
<th>$L^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>201.5</td>
<td>5.93</td>
<td>59.9</td>
</tr>
<tr>
<td>SD</td>
<td>28.6</td>
<td>0.14</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 1. Physical and quality measurements of broiler breast fillets (n = 36) at 24 h postmortem
upon buffer pH. Using chicken breast and leg muscles, Xiong and Brekke (1991) demonstrated that extractability of salt-soluble proteins from isolated myofibrils was lowest at pH 5.5. McCready and Cunningham (1971) observed that salt-soluble protein extraction from chicken muscle increased with buffer pH from 5 to 6.5, but remained unchanged from pH 6.5 to 7. By using extraction buffers with a broad pH range (pH 5.4 to 9), the current study demonstrated that extractability of chicken muscle proteins is similar in neutral pH and high pH buffers. The extractability of myofibrillar proteins is also pH sensitive in red meat. In beef and buffalo muscle, the extractability of salt-soluble proteins peaks at pH 6 (Lan et al., 1993; Thomas et al., 2007). In pork muscle, however, Lan et al. (1993) did not observe a difference in salt-soluble protein extractability with buffers ranging from pH 5.5 to 6.5, but Munasinghe and Sakai (2004) observed an increase in protein extractability as buffer pH increased from 6 to 6.5. Data from the current study suggest that extractability of salt-soluble proteins from chicken meat peaks at greater pH levels than in red meat. Species differences in the pH sensitivity of myofibrillar protein extractability are likely due to inherent differences in muscle fiber type.

Neither the salt-soluble (P = 0.9088) nor water-soluble (P = 0.3252) protein extracts exhibited a significant pH by aging interaction effect on total protein concentration, indicating that postmortem aging did not influence the pH sensitivity of the extractions. Postmortem aging, however, influenced the total amount of protein that could be extracted from the fillets (Figure 2). Protein concentration of the salt-soluble extracts were similar on d 1 and 8 postmortem but lower (P < 0.0001) on d 5. Protein concentrations of the water-soluble extracts were similar on d 1 and 5, but increased (P < 0.0001) on d 8 postmortem. These data suggest that postmortem aging of chicken breast muscle influences protein extractability. Aging-related alterations in protein extractability were likely due to the changes in protein molecular size, conformation, and inter- and intramolecular bonds that occur with postmortem aging. Further research is needed, however, to determine if aging-related changes in extraction influence the functionality characteristics of the proteins contained within the extracts.

During postmortem aging of meat, endogenous protease systems degrade cytoskeletal and myofibrillar proteins (Huff-Lonergan et al., 2010). In broiler breast meat, it has been shown that proteolysis and postrigor tenderization are nearly complete within the first 72 h postmortem (Tomaszewksa-Gras et al., 2011). Similarly, Lee et al. (2009) demonstrated that the change in shear force of broiler breast fillets with postdeboning aging from 2 to 6 d was minimal, but did observe that measures of water-holding capacity and color continued to progressively change with aging up to 6 d. These observations suggest that whereas postmortem proteolysis and tenderness were nearly complete before 72 h postmortem, other yet to be defined physicochemical changes were still occurring in the muscle with extended aging. Thus, although it is possible that the aging-related changes in the protein contents of the salt-soluble extracts in the current study reflect low
levels of both degradation of intact proteins and the simultaneous appearance of degradation products, further research is needed to explain the mechanism by which extended postmortem aging of breast muscles influences protein extractability.

Past data on the effect of postmortem aging on myofibrillar protein solubility are not consistent. Although the postmortem time frame is different due to species differences, several previous studies have observed that salt-soluble protein extractability first decreases and then increases with postmortem aging, similar to the current study. Samejima et al. (1992) observed that myofibrillar protein extractability decreased from 0 to 3 d postmortem and increased from 3 to 7 d in myofibrils from rabbit skeletal and pork cardiac muscle. Similarly, Lan et al. (1993) observed a decrease in salt-soluble protein extraction from 0 to 7 d postmortem and a slight increase from 7 to 14 d in beef and pork. In buffalo muscle, aging from 0 to 15 d postmortem decreased the extractability of salt-soluble proteins (Thomas et al., 2007). Other studies, however, observed that postmortem aging increased myofibrillar protein extractability in beef and rabbit (Chaudhry et al., 1969; Bowker et al., 2008a). Using chicken breast muscle, Li-Chan et al. (1986) observed that a greater abundance of salt-soluble proteins could be extracted from samples at 7 d postmortem than at 0 or 2 d. These varied findings suggest that the effects of postmortem aging on muscle protein solubility are largely dependent on species, aging treatments, and specific extraction conditions.

Whereas sarcoplasmic proteins are known to be susceptible to denaturation early postmortem due to rapid postmortem glycolysis, the effects of extended postmortem aging on sarcoplasmic protein solubility are less clear. A decrease in sarcoplasmic protein solubility with aging has been observed in beef (Bowker et al., 2008b). In pork, however, Boles et al. (1992) observed day-to-day fluctuations in sarcoplasmic protein solubility but could not identify a clear trend with aging from 0 to 7 d postmortem. The endogenous proteases typically associated with meat aging are not known to degrade sarcoplasmic proteins. Thus, it is unlikely that the elevated protein concentrations of water-soluble extracts observed at d 8 were due to direct proteolysis of sarcoplasmic proteins. Further research is needed to determine the cause of the observed increase in water-soluble protein extraction with postmortem aging of breast fillets.

The effects of buffer pH and postmortem aging on the total protein concentrations of the salt-soluble and water-soluble extracts reflect the cumulative effect of these factors on the extractability of the numerous individual proteins within each type of extract. It was hypothesized that extraction buffer pH and aging of chicken muscle would not only influence the total amount of protein extracted from chicken breast fillets but also the relative proportions of the different proteins within the extracts. The intrinsic solubility of an individual protein in an aqueous buffer is dependent upon the distribution of hydrophilic and hydrophobic amino acids on the surface of the protein. Thus, due to differences in amino acid composition and conformation, the various proteins contained in muscle tissue likely have different extractability characteristics. Gel electrophoresis was used to quantify the protein profiles of the muscle extracts. For the salt-soluble extracts, the staining intensities of 15 protein bands ranging from 17 to 200 kDa were quantified (Figure 3). For the water-soluble extracts, 17 protein bands ranging from 21 to 225 kDa were quantified (Figure 4). The salt-soluble extracts contained primarily myofibrillar and cytoskeletal proteins, whereas the water-soluble extracts contained primarily metabolic enzymes.

Data from this study support the hypothesis that extraction buffer pH and postmortem aging influence the protein profiles of the muscle extracts from chicken breast fillets. Table 2 shows proteins that were influenced by extraction buffer pH. Overall, the relative abundance of band 1 (myosin heavy chain), band 5 (80 kDa), and band 13 (troponin-I) in the salt-soluble extracts increased \((P < 0.0001)\) with buffer pH. For bands 1, 5, and 9 extractability was drastically lower at pH 5.4 but relatively unchanged from pH 6.4 to 9. Abundance of salt-soluble band 6 (60 kDa) and band 8 (47 kDa) was highest at pH 5.4. Abundance of band 4 (90 kDa) within the salt-soluble extracts increased from pH 5.4 to 6.4, but then decreased from pH 6.4 to 9. These findings are consistent with past research.
showing that the proportions of myofibrillar proteins in salt-soluble extracts are altered at low buffer pH in pork and rabbit muscle (Samejima et al., 1992). Within the water-soluble extracts, abundance of band D (glycogen phosphorylase), band H (pyruvate kinase), band M (glyceraldehyde phosphate dehydrogenase), band N (lactate dehydrogenase), and band O (phosphoglycerate mutase) increased with extraction buffer pH. For both the salt- and water-soluble fractions, differential extraction of individual proteins due to buffer pH was likely due to pH-induced changes in protein conformation and denaturation.

Table 3 shows the effects of postmortem aging on the electrophoretic profiles of the protein extracts. Salt-soluble band 13 (troponin-I) and band 14 (troponin-C) decreased with aging. Overall, the SDS-PAGE profiles of the salt-soluble extracts exhibited limited proteolysis due to postmortem aging. Although the 30 kDa breakdown product of troponin-T was visible in the salt-soluble extracts from pH 6.9 to 8.0, the abundance was relatively low in both d 1 and 8 samples and was not quantified. Similarly, Li-Chan et al. (1986) observed only minor changes to the SDS-PAGE profiles of salt-extractable proteins from chicken breast muscles that were sampled at 0, 2, and 7 d postmortem. The lack of a strong aging effect between 1 and 8 d samples could be due to the fact that the majority of proteolysis has already occurred by the first sampling time at 24 h postmortem (Li et al., 2012).

Seven protein bands from the water-soluble extracts exhibited a significant postmortem aging effect (Table 3). The relative abundance of band G (phosphoglucomutase) and band O (phosphoglycerate mutase) decreased with aging from d 1 to 8 postmortem, whereas the abundance of band I (phosphoglucose isomerase), band J (enolase), band K (creatine kinase/phosphoglycerate kinase), band L (aldolase), and band M (glyceraldehydes phosphate dehydrogenase) increased with aging. These data indicate that the increase in the protein concentrations of water-soluble extracts on d 8 was primarily due to aging-related increases in the solubility of the major glycolytic enzymes. Past studies using beef and pork have also observed aging related changes in the abundance of various metabolic enzymes in the sarcoplasmic protein fraction (Okayama et al., 1992, 2003; Okumura et al., 2003; Bowker et al., 2008b; Di Luca et al., 2011). Differences in the magnitude and direction of the aging-related changes in specific sarcoplasmic proteins between the current and previous studies are likely related to species differences as chicken breast muscle is comprised of nearly 100% type IIB proteins.
muscle fibers, which have a high content of glycolytic enzymes.

Although the total protein concentrations of the extracts did not exhibit significant pH by aging interaction effects, for 3 salt-soluble proteins and 1 water-soluble protein the pH sensitivity of the extraction was influenced by postmortem aging (Figure 5). Within the salt-soluble extracts, the abundance of band 3 (α-actinin) increased with extraction buffer pH in d 1 samples but decreased in d 8 samples (Figure 5a). The abundance of band 10 (troponin-T) similarly increased with extraction buffer pH in both d 1 and 8 samples, but at pH 8 and 9 the abundance was lower in d 8 extracts (Figure 5b). Aging influenced the abundance of salt-soluble band 15 (myosin light chain 2) at extraction buffers pH 6.4 to 7.5 (Figure 5c). With extraction at low and high pH levels, however, abundance of band 15 was similar between d 1 and 8. In the water-soluble extracts, the abundance of band C (110 kDa) similarly increased with extraction buffer pH in both d 1 and 8 samples, but at pH 6.9 and 7.2 the abundance was greater in d 8 samples (Figure 5d). Overall, these data suggest that pH-related changes in muscle protein extractability and functionality may be influenced by postmortem aging.

Alterations to the protein contents and profiles of the extracts in this study could have been partially due to aging and pH induced protein shifts between the 2 fractions upon extraction. Several reports on beef have suggested that proteins of myofibrillar origin accumulate in the sarcoplasmic fraction or drip extract with postmortem aging (Xiong and Anglemier, 1989; Kolczak et al., 2003). These protein bands were thought to be aging-related degradation products of high molecular weight myofibrillar proteins that were more water-soluble than the intact proteins. Similarly, it is possible that some bands in the electrophoretic profiles of the water-soluble extracts in the current study may have been of myofibrillar origin. More research is required, however, to determine if the presence of high molecular weight myofibrillar proteins in the sarcoplasm-
mic extracts would lead to enhanced functionality characteristics. It is also likely that sarcoplasmic proteins were present in the salt-soluble extracts. Denaturation of sarcoplasmic proteins can reduce their solubility and cause them to adhere to myofibrils upon isolation (Bendal and Wismer-Pedersen, 1962). In particular, glycogen phosphorylase has been shown to undergo this phenomenon in muscle that has undergone a rapid postmortem glycolysis (Fischer et al., 1979; Pietrzak et al., 1997; Zhu et al., 2011). The presence of sarcoplasmic proteins in the salt-soluble extracts was confirmed by Western blot analyses (data not shown) indicating that both band 4 in the salt-soluble extracts and band D in the water-soluble extracts were indeed glycogen phosphorylase. Blot analyses of both types of extracts demonstrated that as the pH of the extraction buffer decreased below 7.2, there was a decrease in glycogen phosphorylase abundance in the water-soluble extracts and a simultaneous increase in the salt-soluble extracts.

Using a basic research approach, this study found that the total amount of protein extracted from normal quality broiler breast fillets as well as the myofibrillar and sarcoplasmic protein profiles of the extracts can be influenced by extraction pH and postmortem aging of the muscle. Overall, this fundamental study demonstrated that the extractability of myofibrillar and sarcoplasmic proteins from broiler breast muscles was influenced by extraction at slightly acidic to neutral pH levels (5.4 to 7.2) and by extended postmortem aging up to 8 d postmortem. Although higher pH levels (7.2 to 9.0) affected the extractability of several individual water-soluble and salt-soluble proteins, the higher pH buffers had minimal impact on the total amount of protein that could be extracted from the breast fillets in the current study. Aging breast fillets up to 8 d postmortem did not influence the pH sensitivity of the total protein extraction but aging did differentially influence the extractability of individual myofibrillar and sarcoplasmic proteins. Further research is needed, however, to decipher the underlying mechanisms that caused the aging and pH-related changes in myofibrillar and sarcoplasmic protein extraction from breast fillets and to determine how subtle shifts in the composition of protein extracts influence protein functionality characteristics important in the development of processed poultry products and functional food ingredients.

**REFERENCES**


