

Dynamic Gelling Properties of Myofibrillar Protein from Skeletal Muscles of Different Chicken Parts[†]

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The rheological characteristics of myofibrillar protein from four parts of chicken carcasses were compared. During thermal gelation, pectoralis major and pectoralis minor proteins exhibited a single loss modulus (G'') peak (45 °C) and two storage modulus (G') peaks (50 and 60°). The final G' value as well as the gel penetration strength decreased with increasing pH from 5.87 to 6.53. These gelling parameters were lower for pectoralis minor protein than for pectoralis major protein. Thigh and drumstick proteins produced similar viscoelastic patterns during gelation, showing a G'' peak around 48 °C and a G' peak around 51 °C. Compared to pectoralis gels, thigh and drumstick gels were substantially less elastic and more sensitive to pH within 50–60 °C. The major disparities in protein gelling behavior among the different chicken parts appeared to be relevant to the specific distribution of white and red fibers within the muscles.

INTRODUCTION

Consumption of poultry meat has increased substantially over the past decade. In particular, current consumer demands for light meat from chicken breast, in comparison to dark meat from leg or thigh, remain very high. Dark chicken meat is generally less desirable, among other reasons, because of its dull color and relatively high fat and cholesterol content. Therefore, much of chicken thigh and drumstick meat (compared to only a small proportion of breast meat) is further processed to enhance its economic value and marketability.

Several studies have demonstrated disparities in functionality and processing quality between light and dark meat, including both chicken and turkey. Froning and Norman (1966) and Maesso et al. (1970) found light chicken meat to have superior texture and binding strength over dark chicken meat in restructured products. Richardson and Jones (1987) also noted a greater swelling ability and protein extractability for light turkey meat than for dark turkey meat. However, conflicting observations were reported by Amato et al. (1989) in a system where pH of light and dark turkey meat was not standardized. When the pH of both meat types was adjusted to common values, dark meat "gels" remained slightly stronger (shear stress), or showed no difference, than light meat "gels" (Daum-Thunberg et al., 1992). In an attempt to explain the textural and rheological variations between processed light and dark meat, a number of model studies using isolated myofibrillar proteins have been conducted. Most investigations have indicated that myofibrillar protein plays a significant role in causing product variations among different muscle types (Asghar et al., 1984; Foegeding, 1987; Morita et al., 1987; Xiong and Brekke, 1991; Xiong, 1992).

Skeletal muscles located at different parts of chicken carcasses consist of fibers that differ in histochemical types. Slow-twitch type I and type IIA and fast-twitch type IIB, which are also known as β -red, α -red, and α -white (Ashmore, 1974), are three major fiber types found in

skeletal muscles. Pectoralis muscle is rather homogeneous in type IIB white fibers (Rosser and George, 1986), whereas thigh and drumstick muscles, both of which are comprised of numerous individual muscles, have a preponderance in type I and type IIA red fibers (George and Berger, 1966; Suzuki et al., 1985). Asghar et al. (1984) reported that myosin from chicken pectoralis profundus muscle (white) generally formed more rigid (shear modulus) gels than myosin from gastrocnemius muscle (red) under identical pH, temperature, and ionic conditions. Similar differences were observed between myosins of whole chicken breast and leg muscles (Morita et al., 1987). Xiong and Brekke (1990, 1991) noted that more complex protein systems, e.g., myofibrils and salt-soluble protein from chicken whole breast muscle, also produced stronger (penetration strength) gels than those from whole leg muscle. These results, as well as those from numerous other studies summarized in a recent review (Xiong, 1994), implicate that gelling properties of myofibrillar proteins are influenced by the distribution of specific muscle fiber types.

Due to differences in glycolytic metabolism between red and white fibers, the ultimate pH of red muscle generally differs from that of white muscle. Several recent studies have shown that pH can greatly influence the physical and functional behavior of muscles during thermal processing (Richardson and Jones, 1987; Daum-Thunberg et al., 1992; Barbut and Mittal, 1993). Daum-Thunberg et al. (1992) demonstrated that the initial pH of turkey light or dark meat dictated the rheological and water-holding properties of salted batters. However, adjustment of the light meat pH to the dark meat pH, or vice versa, altered the rheological characteristics of the meat emulsion.

The purpose of this study was to evaluate the functional properties of myofibrillar proteins from four different muscle groups of chicken carcasses (pectoralis major and minor from the breast portion and thigh and drumstick from the leg portion) by comparing their gelling behaviors using dynamic and static rheological measurements under various pH conditions.

MATERIALS AND METHODS

Preparation of Myofibrils. Three replicate groups of 24 chickens each (6–8 weeks of age) of commercial strain crosses were slaughtered at the University of Kentucky Poultry Research

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Facility. Carcasses were kept in ice for 24–48 h postmortem before use. Myofibril isolation was performed at 0–2 °C using combined pectoralis major (*P. major*), pectoralis minor (*P. minor*), thigh, or drumstick muscles from four randomly selected carcasses, and the isolation was repeated three times (replications) on different days (Xiong, 1993). The isolation buffer (pH 7.0) was composed of 0.1 M NaCl, 50 mM phosphate ($\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$), and 1 mM sodium azide (NaN_3) as antimicrobial agent. After isolation and purification, myofibrils were suspended in 8 volumes (w/v) of 0.1 M NaCl solution and then divided into four equal portions which were subsequently titrated to, respectively, pH 5.87, 6.19, 6.38, and 6.53 using 0.1 N HCl prior to centrifugation. These pH values were selected because they represented the respective mean pHs found in fresh *P. major*, *P. minor*, thigh, and drumstick muscles of these chickens. Protein concentration was determined using the biuret method (Gornall et al., 1949) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as the standard.

Determination of Muscle pH. For each muscle type, pooled muscle samples from five chickens were employed for pH measurement; this was done with four replications (total of 20 chickens). In each replication, triplicate minced muscle samples (2 g each) were homogenized for 15 s in 20 mL of 0.1 M NaCl solution using a PT 10/30 Polytron homogenizer equipped with a low-foam PTA 20TS generator (Brinkmann Instruments Inc., Westbury, NY) at speed 6. The pH of the homogenates was determined using a combination probe connected to a Model 220 Corning pH meter.

Electrophoresis. Protein composition of myofibril preparations was determined by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) (Xiong, 1993) in a Model SE 250 Mighty Small II slab gel electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). A 3% acrylamide stacking gel and a 10% acrylamide resolving gel were used. Protein bands were tentatively identified on the basis of their relative mobility in comparison with published literature (Hay et al., 1973; Porzio and Pearson, 1977).

Protein Extractability. Three replicated experiments were conducted using freshly prepared myofibril samples. Extraction of protein from myofibrils was carried out in 0.6 M NaCl, 50 mM phosphate, at pH 5.87, 6.19, 6.38, and 6.53 as described elsewhere (Xiong and Brekke, 1991). Protein extractability was defined as protein concentration of supernatant (after centrifugation) divided by protein concentration of the original suspension times 100.

Dynamic Viscoelasticity Measurements. Myofibril pellets at specific pHs were suspended in 50 mM sodium phosphate buffers at the same pHs and subsequently stored at 0 °C for 20 h to ensure maximum protein extractability (Xiong and Brekke, 1991). The buffers contained predetermined amounts of NaCl to produce a final NaCl concentration of 0.6 M and a protein concentration of 20 mg/mL. Nondestructive, oscillatory measurements of the protein suspensions during gelation were performed using a Bohlin VOR rheometer (Bohlin Instruments, Inc., Cranbury, NJ) as described by Xiong (1993). Thermal sol-to-gel transformation was induced by heating the protein suspension from 20 to 74 °C at 1 °C/min; sample temperature was verified using a thermocouple attached to the lower plate. Gelation was monitored by shearing the sample at a fixed frequency of 100 mHz with a maximum strain of 0.02. Rheological properties of the myofibrillar gelling system (sol and gel) were described in terms of storage modulus (G' , the elastic component) and loss modulus (G'' , the viscous component).

Gel Penetration Test. Thermally induced myofibrillar gels (20 mg/mL protein in 0.6 M NaCl, 50 mM phosphate at specific pHs) were prepared in 16.5-mm (inner diameter) × 80-mm (length) glass vials by heating the protein suspensions from 20 to 70 °C at 0.75 °C/min in a waterbath (Xiong, 1993). Gel penetration strength (force) was measured in a Model 4301 Instron universal testing device (Instron Corp., Canton, MA) as previously described (Xiong, 1993).

Statistical Analysis. Protein extractability and gel viscoelastic properties were analyzed using general ANOVA which included replication effect (Statistix 3.5, Analytical Software Inc., St. Paul, MN). The effects of pH and muscle type were both found significant ($P < 0.05$). Thus, differences in protein

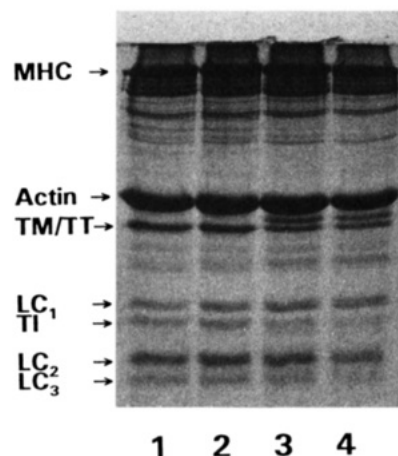


Figure 1. SDS–PAGE of myofibrillar protein from chicken pectoralis major (lane 1), pectoralis minor (lane 2), thigh (lane 3), and drumstick (lane 4) muscles. MHC, myosin heavy chain; TM, tropomyosin; TT, troponin-T; TI, troponin-I; LC₁, LC₂, and LC₃, myosin light chains 1, 2 and 3, respectively. A 20- μ g protein sample (in 10% β -mercaptoethanol) was loaded to each lane.

extractability and gel parameters due to pH and muscle type were separated by the least significant difference test (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

Muscle pH and Protein Composition. The pH ranges for *P. major*, *P. minor*, thigh, and drumstick muscles were, respectively, 5.80–5.94, 6.14–6.24, 6.34–6.43, and 6.45–6.61, with mean values of 5.87, 6.19, 6.38, and 6.53. These pH values were slightly higher than those reported by Amato et al. (1989), but similar in trend (i.e., drumstick > thigh > breast). The four chicken muscles studied can be placed in two groups, on the basis of their myofibrillar protein composition. The first group encompasses *P. major* and *P. minor* (Figure 1, lanes 1 and 2), both of which displayed a single protein band in the tropomyosin/troponin-T area and exhibited a relatively high intensity in troponin-I and myosin light chain bands (Figure 1). Thigh and drumstick (Figure 1, lanes 3 and 4) comprise the second group, which showed an appreciable amount of myosin light chain 1 and light chain 2, but was relatively deficient in light chain 3. Additionally, this myofibril group had two bands in the tropomyosin/troponin-T area. These results were consistent with previous observations on chicken whole breast and whole leg muscles (Hay et al., 1973; Obinata et al., 1979; Xiong and Brekke, 1991), and the compositional differences between the two groups of myofibrils were typical of white and red fibers (Young and Davey, 1981).

Protein Extractability. Protein extractabilities of *P. major* and *P. minor* myofibrils were essentially identical, and to a large extent, both appeared independent of the pH within the range 5.87–6.53 (Table 1). Thigh myofibrils were more sensitive to pH than were drumstick myofibrils, showing an increase in extractability from 49.3% at pH 6.53 to 59.0% at pH 6.19. Overall, however, both thigh and drumstick myofibrils contained a similar amount of extractable protein. Protein extractability of the four muscle samples also can be separated into two groups, i.e., either greater than 70% (for *P. major* and minor) or less than 60% (for thigh and drumstick). Three fiber types, i.e., slow, intermediate, and fast (or β -red, α -red, and α -white), and a number of their subgroups are present in chicken leg muscles, with the slow β -red type being prevalent (Aberle et al., 1979; Suzuki et al., 1985). In

Table 1. Effect of pH on Extractability of Myofibrillar Protein from Chicken Breast and Leg Muscles

pH	protein extractability (%)			
	P. major	P. minor	thigh	drumstick
5.87	70.8 ^b (2.0)	71.7 ^{ab} (0.5)	55.3 ^c (0.8)	54.6 ^{cd} (1.4)
6.19	72.8 ^{ab} (1.0)	74.0 ^{ab} (2.3)	59.0 ^c (1.7)	52.5 ^d (0.9)
6.38	75.6 ^a (2.3)	74.2 ^a (1.7)	51.7 ^d (0.2)	53.5 ^{cd} (0.1)
6.53	70.8 ^b (1.9)	72.9 ^b (2.1)	49.3 ^d (0.5)	51.6 ^d (0.9)

^{a-d} Means within the same row or column bearing different superscripts differ significantly ($P < 0.05$). Values in parentheses are standard errors of the means (three replicates with duplicate measurements).

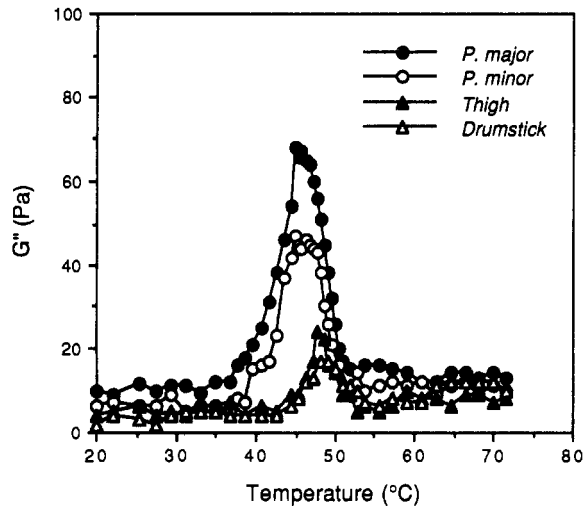


Figure 2. Changes in loss modulus (G'') of chicken white and red muscle myofibrillar protein suspensions during thermal gelation. Gels were induced by heating the myofibril suspensions (20 mg/mL protein in 0.6 M NaCl, 50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, pH 5.87) from 20 to 74 °C at 1 °C/min.

contrast, chicken pectoralis muscles are comprised almost exclusively of fast α -white fibers (Rosser and George, 1986). It is plausible that ultrastructural variations and differences in postmortem proteolytic susceptibility between white and red myofibrils (Hay et al., 1973) could have contributed to the disparity in protein extractability. Parsons and Knight (1990) also compared the extractability of myosins from red and white bovine and rabbit myofibrils and found that myosin from slow myofibrils was more resistant to extraction than myosin from fast myofibrils in salt solution.

Dynamic Viscoelastic Properties. Heating induced a major peak in loss modulus (G'') for chicken pectoralis and leg myofibril suspensions (Figure 2). For pectoralis proteins, G'' began ascending at about 35–39 °C, indicating the onset of protein denaturation (unfolding) which enhanced the viscous component of the protein suspension. Despite differences in magnitude, P. major and P. minor exhibited similar changes, reaching maximum G'' values around 45 °C. Thigh and drumstick myofibril suspensions produced similar G'' peaks; however, these peaks were smaller and occurred at a higher temperature (around 48 °C) than did breast myofibril suspension peaks (Figure 2). The decline in G'' probably resulted from the formation of protein networks that are less viscous in nature. While the higher temperature for G'' peak may suggest that leg myofibrils or their constituents were more stable than breast myofibrils, the low magnitude in leg G'' probably resulted from the lower protein solubility and less capa-

bility of the leg sample to form a hydrodynamic mass at the initial stage of gel formation.

Corresponding to the marked reduction in G'' shown above, storage modulus (G') of P. major and P. minor increased rapidly from 45 to 50 °C (Figure 3), which was evidence of the transformation from a viscous sol to an elastic gel network. However, this G' increase was followed immediately by a sharp decline that reached a minimum around 53–56 °C (Figure 3). Thus, thermally induced protein networks, presumably via myosin denaturation, were dynamic in nature and unstable at temperatures greater than 53–56 °C. The G' increased abruptly at higher temperatures, exhibiting a shoulder peak around 60 °C. The viscoelastic transitions probably resulted from an initial loose cross-linkage among protein molecules, followed by a redistribution of intermolecular forces or formation of new bonds to produce a more permanent protein network structure.

The exact magnitude of G' at peak or at the end of heating (74 °C), the temperatures for G' transition, as well as the peak/shoulder resolution, were influenced by the specific pH and muscle type (Figure 3; Table 2). In general, a pH close to 6.0 favored gel network formation and produced more elastic gels than a higher pH, and this result was in good agreement with previous observations (Wang et al., 1991). The viscoelastic curves of P. minor protein virtually duplicated the curves of P. major with the G' peak occurring at identical temperatures at respective pHs (Table 2). However G' values of P. minor were less than those of P. major ($P < 0.05$). P. major and P. minor muscles are both homogeneous in white fibers and additionally, myofibrils from both muscles had an identical protein composition (Figure 1) and were equally extractable (Table 1). Hence, the most logical explanation for the difference in G' magnitude between the two types of myofibrillar protein would be that the exact anatomical location (carcass part) and perhaps the initial muscle pH value (5.87 for P. major and 6.19 for P. minor) could have an effect on the protein functionality.

Within the red muscle group, thigh and drumstick produced almost identical viscoelastic patterns during thermal gelation (Figure 4). Similar to pectoralis samples, leg muscle proteins formed most elastic gels at pH 5.87. However, the marked decrease in G' immediately preceding 51 °C was less pronounced for leg samples particularly at low pHs. Moreover, leg myofibrillar gels were devoid of the shoulder transition which occurred in breast gels around 60 °C. Furthermore, the magnitude of G' for leg gels was less than 50% that for breast gels, indicating that leg protein was less capable of forming a highly elastic gel network. Note that under identical gelling conditions, the temperature difference between G' and G'' peaks for leg myofibrils (at pH 5.87) was about 3 °C, compared to 5 °C for breast myofibrils (based on Figures 2–4; Table 2). Thus, it is conspicuous that aggregation of leg myofibrillar proteins followed protein unfolding more closely than did aggregation of pectoralis proteins. This might partially explain the smaller magnitude of G' for leg than for breast proteins, because it appears that insufficient time was allowed for the unfolded leg protein molecules to rearrange to form ordered aggregates, which is essential for producing a highly elastic gel matrix (Hermansson, 1978).

Overall, these results were consistent with most previous findings that myofibrillar proteins from white muscle (breast) fibers, whether singly (myosin and subfragments) or in complex (actomyosin, myofibrils, salt-soluble protein), formed more elastic and rigid gels with a stronger water-binding property than myofibrillar proteins from

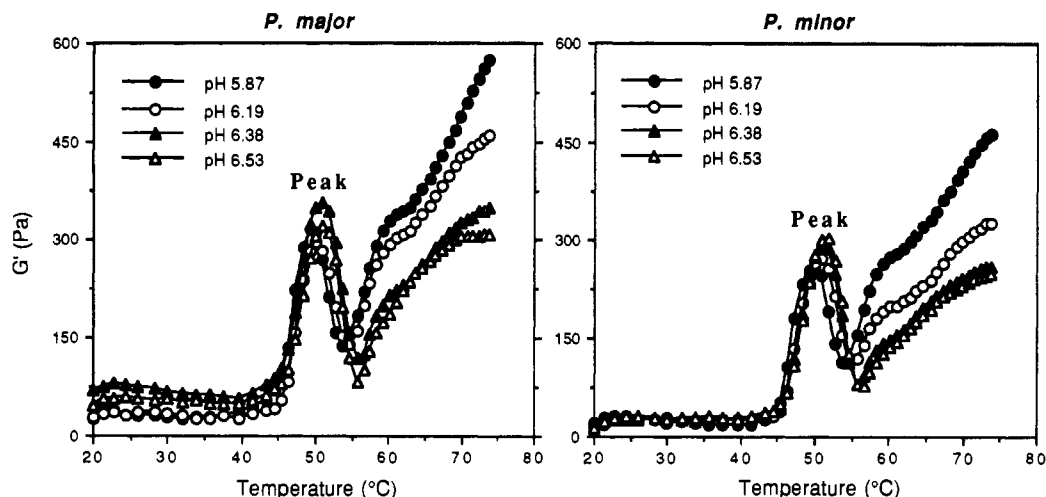


Figure 3. Changes in storage modulus (G') of chicken white muscle myofibrillar protein suspensions at various pHs during thermal gelation. Gels were induced by heating the myofibril suspensions (20 mg/mL protein in 0.6 M NaCl, 50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$) from 20 to 74 °C at 1 °C/min.

Table 2. Shear Storage Modulus and Temperature Maximum at Peaks for Myofibrillar Suspensions during Gelation*

pH	T_p				G'_p				G'_t			
	Pmaj	Pmin	T	D	Pmaj	Pmin	T	D	Pmaj	Pmin	T	D
5.87	49.4 ^b (0.3)	49.0 ^b (0.3)	51.0 ^a (0.0)	51.3 ^a (0.7)	305 ^{ab} (33)	266 ^c (3)	111 ^d (25)	89 ^d (9)	629 ^a (102)	458 ^b (8)	198 ^c (24)	226 ^c (24)
6.19	49.9 ^b (0.5)	50.3 ^b (0.6)	51.5 ^a (0.1)	51.4 ^a (0.1)	349 ^{ab} (29)	271 ^c (1)	98 ^d (23)	91 ^d (32)	462 ^{ab} (89)	320 ^c (7)	108 ^d (5)	144 ^d (29)
6.38	50.3 ^{ab} (0.6)	50.8 ^{ab} (0.8)	51.5 ^a (0.5)	51.7 ^a (0.6)	365 ^a (42)	289 ^b (1)	82 ^d (35)	96 ^d (8)	355 ^{bc} (95)	244 ^d (15)	67 ^e (10)	101 ^e (4)
6.53	50.2 ^b (0.8)	50.5 ^{ab} (1.0)	51.5 ^a (0.0)	51.1 ^{ab} (0.4)	352 ^a (61)	269 ^{bc} (17)	97 ^d (32)	82 ^d (36)	299 ^c (67)	198 ^d (50)	86 ^f (1)	82 ^f (8)

* T_p = temperature maximum at peaks (indicated in Figures 3 and 4); G'_p , G'_t = storage moduli at the peaks and at the end of heating (74 °C), respectively. Chicken parts: pectoralis major (Pmaj), pectoralis minor (Pmin), thigh (T), and drumstick (D). ^{a-f} Means within the same row or column in each parameter group, bearing no common superscript, differ significantly ($P < 0.05$). Values in parentheses are standard errors of the means (two replicates with duplicate measurements).

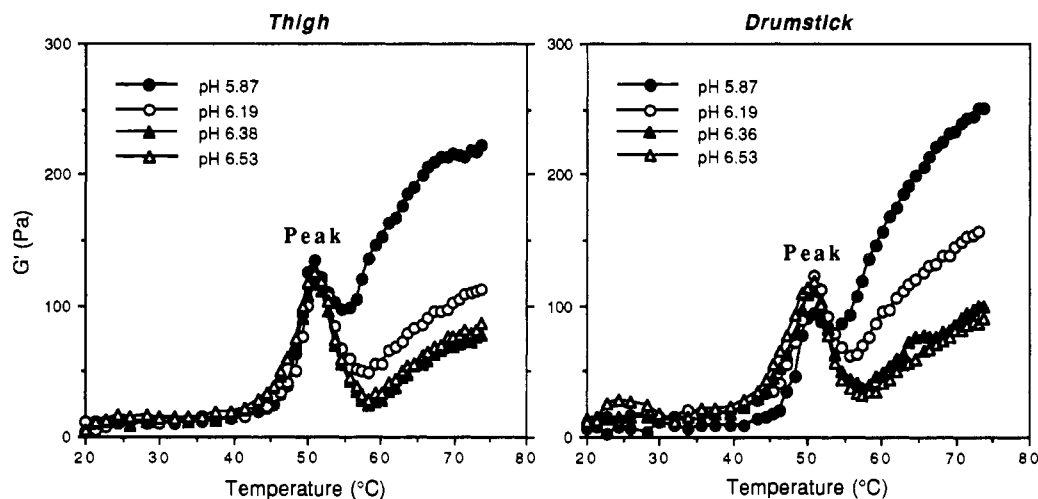


Figure 4. Changes in storage modulus (G') of chicken red muscle myofibrillar protein suspensions at various pHs during thermal gelation. Gels were induced by heating the myofibril suspensions (20 mg/mL protein in 0.6 M NaCl, 50 mM $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$) from 20 to 74 °C at 1 °C/min.

red muscle (leg) fibers of the poultry species (Asghar et al., 1985; Foegeding, 1987; Morita et al., 1987; Samejima et al., 1989; Xiong and Brekke, 1991; Wang et al., 1990; Choe et al., 1991; Xiong, 1992). Myosin of chicken breast muscle has a different morphology from myosin of chicken leg muscle, is able to produce longer filaments, and thermally aggregates more readily than myosin from chicken leg muscle (Morita et al., 1987; Xiong, 1992). These physicochemical properties of myosin isoforms seem to influence texture and ultrastructure of gels, thereby affecting the viscoelastic attributes of the gels.

Gel Penetration Strength. Table 3 summarizes the Instron testing results for myofibril gels prepared at various pH values. Gels formed at pH 5.87 were generally more resistant to penetration, requiring a greater force for structural failure, than gels produced at higher pHs. In spite of the greater ($P < 0.05$) gel strength for P. major than for P. minor at pH 6.19 or higher, both muscles formed stronger gels than thigh and drumstick muscles. For the same pHs, gels formed within the red muscle group were equally rupturable. These results support the dynamic gelling data (G' , G'') shown previously, suggesting that

Table 3. Penetration Force Required To Disrupt Myofibrillar Gels

pH	penetration forces (N)			
	P. major	P. minor	thigh	drumstick
5.87	1.14 ^a (0.25)	0.78 ^{ac} (0.34)	0.40 ^c (0.03)	0.32 ^c (0.12)
6.19	0.74 ^b (0.03)	0.43 ^c (0.14)	0.22 ^d (0.01)	0.20 ^{cd} (0.06)
6.38	0.87 ^b (0.00)	0.47 ^c (0.16)	0.17 ^d (0.03)	0.16 ^d (0.06)
6.53	0.66 ^b (0.04)	0.38 ^c (0.14)	0.19 ^d (0.01)	0.13 ^d (0.04)

^{a-d} Means within the same row or column bearing different superscripts differ significantly ($P < 0.05$). Values in parentheses were standard errors of the means (two replicates with triplicate measurements).

static and dynamic properties of myofibrillar protein gels were similarly affected by intrinsic as well as extrinsic factors. Certainly, data from the dynamic rheological test (G' and G'') would be more meaningfully comparable with results from the penetration test, if gels analyzed by both tests were maintained at the same temperature.

Conclusions. Functional properties of poultry myofibrillar protein are dictated by the fiber type composition within the muscle and influenced by the pH. The results also support the general idea that white myofibrils are more soluble and are capable of forming more rigid or elastic gels than red myofibrils under the pH and ionic conditions typically encountered in poultry processing. Although unable to reproduce the functionality exhibited by white muscle protein, adjustment of red muscle pH to white muscle pH can improve the protein extractability and gelling ability.

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