# Effect of pH on the Rheological and Structural Properties of Gels of Water-Washed Chicken-Breast Muscle at Physiological Ionic Strength

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Adjustment of pH from 6.4 to neutrality improved gelling ability and water-holding capacity of twice water-washed, minced chicken-breast muscle significantly at physiological ionic strength, at which the majority of the myofibrillar proteins, including myosin, are not soluble. A strain value of 2.2 was obtained at neutral pH. Myofibrils were the main components of the gel network at both pH 6.4 and 7.0; however, the myofibrillar distribution varied with the pH value. At pH 6.4, myofibrils formed a network of localized aggregates leaving large voids between, whereas at neutral pH, an evenly distributed network of myofibrils was formed. In addition, at neutral pH, a network of fine strands was found within the network of myofibrils. The network was much less developed at pH 6.4. The thin and thick filaments within each myofibrillar structure were disorganized at both pH values. The intramyofibrillar spaces were larger at neutral pH than at pH 6.4. It was proposed that adjustment of pH to neutrality increased electrostatic repulsion leading to a more even distribution of the myofibrillar proteins, a key factor responsible for the improved gel strength and water-holding capacity.

## INTRODUCTION

Two of the most important factors that affect gelation of myofibrillar proteins are salt concentration and pH. Much research has led to the conclusion that application of salt (0.3−0.6 M) is the prerequisite for the solubilization, and subsequently the gelation, of myofibrillar proteins (*1, 2*). The extracted salt-soluble proteins are thought to be largely responsible for textural and structural properties of heat-induced gels (*3, 4*). The optimum pH for gelation is often reported as between 5.5 and 7.0, which is dependent on the protein concentration and the species from which the salt-soluble proteins are derived (*5−11*).

Gelling properties of synthetic filaments from pure myosin molecules at reduced salt concentrations have been characterized (*6, 12−15*). The preparation of synthetic filaments involved the solubilization of myosin molecules at high ionic strength (0.6), followed by reduction of ionic strength (0.1−0.2). The optimum pH for gelation of synthetic filaments was about 6.0 (*6, 13, 14*).

Recent studies have demonstrated that elastic gels with high water-holding capacity can be formed directly from water-washed minced muscle at physiological ionic strength (0.15) without first solubilizing myofibrillar proteins at high salt concentrations (*11*). Gelation of these insoluble myofibrillar proteins requires neutral or slightly alkaline pH. The gelling ability decreases significantly as the pH decreases from a neutral to a slightly acidic one (e.g., 6.4). Theories of gel formation of muscle proteins propose that solubilization of myosin is a necessary first step. At neutral or slightly acid pH, myosin is insoluble at physiological ionic strength. The research reported here was carried out to determine how and why good gels could be formed from washed, minced chicken-breast muscle at physiological ionic strength and to explain why a neutral pH was superior to a slightly acid one.

## MATERIALS AND METHODS

**Materials.** Adult chickens were obtained from the Department of Veterinary and Animal Sciences at the University of Massachusetts in Amherst. The birds were sacrificed by carbon dioxide asphyxiation. Breast muscles were taken and packaged in plastic bags and placed on ice immediately. Further processing was carried out within 60 min. Connective tissues were removed to the extent possible, and the remaining muscle was ground through a 5-mm diameter plate and thoroughly mixed by hand for about 2 min in a beaker.

**Preparation of Twice Water-Washed Minced Muscle.** A 20-g portion of the ground muscle tissue was homogenized with 200 mL of cold (4−6 °C) deionized, distilled water in a commercial Waring blender (Waring Products Division, Dynamics Corp. of America, New Hartford, CT) for 60 s, followed by centrifugation at 37000*g* for 20 min. The sediment was washed once again with water of the same volume to obtain twice water-washed minced muscle. Cryoprotectants, including 4% sucrose, 4% sorbitol, and 0.3% tripolyphosphate, were chopped into the twice water-washed minced muscle before storage at −20 °C.

**Preparation of Gels.** Protein gels were prepared from twice water-washed minced muscle with and without cryoprotectants. The protein paste was mixed in a mortar with pestle by hand for 3 min. The mortar was cooled in an ice−

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water bath. The pH was adjusted to 6.4 or 7.0 with 250 mM $Na_2CO_3$ during mixing. The ionic strength was adjusted to 0.15 M with NaCl. The paste was stuffed into cylindrical steel tubes whose ends were sealed with crown-type bottle caps, heated in a water bath at either 90 °C or 72 °C for 30 min, and cooled immediately in ice water. Gels were stored for 24 h at 5 °C before analysis. Variations in moisture content, ionic strength, pH, and heating methods were used as noted.

**Protein Content.** Protein content was measured by the Lowry procedure (*16*) with bovine serum albumin as the standard.

**pH Measurement**. pH was determined using an Orion combination electrode (model 51-57) in conjunction with an Orion pH meter (model 420A, Orion Research Inc., Boston, MA). Protein paste (5 g) was thoroughly dispersed in 50 mL of distilled deionized water (if the sample was the ground muscle tissue or gel, it was blended in water for 1 min). The pH was read by placing an electrode into the suspension and allowing the reading to stabilize. Slight and constant stirring of the protein suspension was provided while the pH was being measured.

**Ionic Strength.** Ionic strength was estimated by measuring the conductivity of a diluted protein solution with a conductivity meter (YSI, Inc., Yellow Springs, OH). The value was expressed in equivalent sodium chloride concentration by comparing the conductivity reading of a protein solution to a standard curve prepared with sodium chloride.

**Moisture Content.** The material was spread thinly on a flat aluminum disk (about 2 g) and placed in a ventilated oven at 105 °C for at least 18 h. The moisture content was determined based on the weight loss after drying.

**Torsion Test of Gels.** The torsion test developed by Hamann (*17*) was used to assess gelation characteristics. Gels were allowed to equilibrate to room temperature (20−25 °C) prior to milling into dumbbell-shaped specimens as described by Lanier et al. (*18*). Specimens had a minimum center diameter of 10 mm, length of 28.7 mm, and end diameter of 18.6 mm. Ends of samples were glued to plastic disks (Piedmont Plastics, Charlotte, NC) with instant "Krazy Glue" (Elmer's Products, Inc., Columbus, OH) and mounted on a torsion apparatus consisting of a Brookfield digital viscometer (model DV-II, Brookfield Engineering Laboratories Inc., Stoughton, MA). The viscometer was operated at 2.5 rpm and the outcome was recorded on a chart recorder. Shear stress and shear strain were calculated using equations provided by Hamann (*17*).

**Water-Holding Capacity of Gels.** Water-holding capacity of a gel was defined as the percentage of the total water that was retained after cooking and pressing [WHC = 100 − (cooking loss + pressing loss)]. Cooking loss was defined as the loss of water after heating at a certain temperature for a certain period of time and expressed as a percentage of the total water (before heating). Gels were cooked at 90 °C for 30 min unless otherwise stated. The cooking loss was calculated based on the measurements of the moisture content before and after heating. Pressing loss was defined as the water loss after a slice of gel with a thickness of 3 mm was pressed under a weight of 3000 g for 1 min. The specimen was sandwiched by five layers (or more) of filter paper of medium porosity (P5, Fisher Scientific, Pittsburgh, PA). Sufficient layers of filter paper were provided to absorb the expressible water. The pressing loss was calculated based on the moisture contents before and after pressing. The cooking and pressing losses were expressed as a percentage of the total water before cooking.

**Dynamic Rheological Measurement**. The dynamic gelling properties of a protein suspension or paste were characterized with a Bohlin Rheometer (Bohlin CS-10, Bohlin Instruments, Cranbury, NJ) using a pair of coaxial cylinders. The diameter of the inner cylinder (Bob) was 25 mm and the internal diameter of the outer cylinder (Cup) was 27.5 mm. A 13-g portion of protein suspension or paste was loaded in the gap between the two cylinders. The top of the specimen was covered with a thin layer of silicon oil to prevent evaporation of water. The instrument was set to the oscillation mode with a fixed frequency of 1 Hz and a maximum shear strain of 0.01.

The protein paste was stored at 5 °C and loaded into the cup that was kept at room temperature. The circulating water in the tank was maintained at 72 °C. The heating water was let into the rheometer after the setup was complete. Data were collected after an equilibrium period of 30 s. Samples were heated at a constant temperature of 72 °C for 20 min. The rheological properties were expressed in terms of the storage modulus (G′, the elastic component), the loss modulus (G″, the viscous component), and the phase angle (δ, the phase shift between input and output).

**Phase Contrast Microscopy.** A phase contrast microscope (Microstar Series 10, American Optical Co., Buffalo, NY) was used to observe the morphological changes of cell segments and myofibril segments. Images were recorded onto a black and white photographic film (Tmax 100, Eastman Kodak Company, Rochester, NY). A green interference filter was inserted into the light path for a better black and white photomicrograph.

**Transmission Electron Microscopy**. Gel samples were prepared from twice water-washed, minced chicken-breast muscle. The washed, minced muscle was stored in plastic bags on ice for approximately 24 h. The final moisture content before heating was adjusted to 81%. The final paste was stuffed in a metal tube with a diameter of 10 mm and cooked at 72 °C for 30 min. Gels were cooled immediately with ice water after heating. Specimens for thin-sectioning were prepared according to Wang and Smith (*19*) with some modifications. Briefly, gels were cut into small disks of 0.5-mm thickness and fixed with 2% (v/v) glutaraldehyde in a buffer of 0.1 M sodium phosphate at pH 7.2. These disks were further trimmed into smaller cubes and postfixed with 1% (w/v) $OsO_4$. The fixed specimens were stained with uranyl acetate ($H_2O$/saturated solution, 1:1) and dehydrated in a series of acetone concentrations (25%, 50%, 75%, and 100%), followed by infiltration with Epon/Araldite (502). Specimens were polymerized at 70 °C for at least 8 h. Thin sections were post-stained with Reynolds lead−citrate solution (*20*) for 2 min and viewed in a JEOL transmission electron microscope (JEM-100S, Tokyo, Japan) at 80 kV.

**Replication and Statistical Analysis.** All the experiments were repeated at least twice, and in each experiment all the analytical measurements were done in duplicate. Results were reported as means ± standard deviations. In figures, the standard deviations were shown with error bars. If no error bars are shown, the standard deviation is smaller than the symbol used. The stress and strain values obtained from protein gels were compared by their mean values between the treatments, and significant differences ($P < 0.05$) were identified using the least significant difference (LSD) procedure (*21*). Micrographs of minced muscle and cooked gels were the typical views obtain from at least three samples. Dynamic viscoelastic measurements were carried out in three replicated experiments and a typical thermal scan was selected.

## RESULTS

**Twice Water-Washed, Minced Chicken-Breast Muscle.** Homogenization in a commercial Waring blender for 1 min disrupted the muscle tissue into segments of myofibrils and bundles of myofibrils with a few scattered cell segments. The pH of this initial homogenate was about 6.2. A typical view of twice water-washed, minced chicken-breast muscle by phase contrast microscopy is shown in Figure 1. Two water washes removed about 24% of the total protein; 23% was removed in the first water wash. The second water wash removed only 1.2% of the total protein. Proteins soluble under these conditions are termed sarcoplasmic proteins. The remaining insoluble fraction is largely the myofibrillar proteins that include myosin and actin, the two main components of muscle proteins (*22*). The moisture content of the twice water-washed, minced chicken-breast muscle was about 79%. After two water
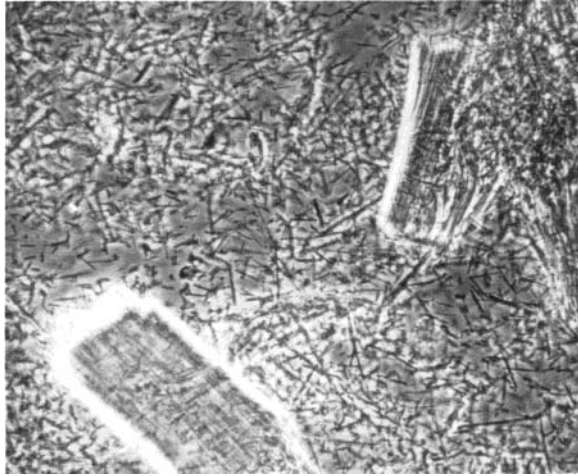
**Figure 1.** Suspension of twice water-washed, minced chicken-breast muscle in a solution of 150 mM NaCl. Preparation of twice water-washed, minced chicken-breast muscle is described in the Materials and Methods section. The image was taken under a phase contrast microscope. The magnification of the image is 190×.

washes (1:10 w/v), the ionic strength was reduced from its physiological value ($\sim$0.15) to approximately 0.7 × 10$^{-3}$. The pH of the twice water- washed minced muscle was about 5.9. Its buffering capacity was greatly reduced by the washing-out of phosphate salts, amino acids, and derivatives, as well as the sarcoplasmic proteins.

**Torsion Test.** For this test, gels were prepared from twice water-washed minced muscle with added cryoprotectants. Their gelling ability at a salt concentration of 150 mM NaCl over the pH range of 6.4 to 7.0 was characterized by the torsion test (Figure 2). Gels showed an increase in stress and strain values with an increase of pH. Both stress and strain values showed significant difference between pH 6.4 and 7.0 ($P < 0.05$). Adjustment of pH from 6.4 to 7.0 raised the stress value from 88 to 130 kPa and the strain value from 1.1 to 2.2. Stress value often responds to the textural strength, whereas strain value is related to the elasticity of gels (*23*). The latter is less affected by moisture content (*24*) and correlates better to the gelling capability of the muscle proteins. A strain value of 1.9 or greater will pass the traditional Japanese double-fold test for acceptable surimi gels (*25*).

**Water-Holding Capacity.** The water-holding capacity of cooked gels with standard cryoprotectants was evaluated by measuring the water losses after cooking and pressing as shown in Table 1. At pH 6.4, the total water loss after cooking and pressing reached 43.8%, which nearly tripled the amount of water loss at pH 7.0 (15.1%). The majority of that water was released at the pressing stage. Its amount increased as the pH decreased from 7.0 to 6.4 (Table 1). The total water loss as a function of pH was not linear. By comparing a pH unit of 0.2 in the range of pH 6.4 to 7.0, the greatest reduction of total water loss was observed from pH 6.6 to 6.8 (15%), and following it was the pH from 6.4 to 6.6 (10%). The least reduction was found between pH 6.8 and 7.0 (2%).

**Protein Concentration.** The effect of protein concentration and pH on dynamic gelling properties is illustrated in Figure 3. Comparisons were made between pH 6.4 and 7.0 ranging from 2 to 10% of protein. All samples were heated at 72 °C for 20 min. At a given protein concentration, the storage modulus developed at neutral pH was higher than that produced at pH 6.4 before, during, and after the heating process. The net changes of the final storage modulus due to the pH adjustment (6.4 → 7.0) as a function of protein concentrations are shown in Figure 4. The protein concentration is expressed as the grams of protein in 100 g of water. The net change of the storage modulus increased gradually until the protein concentration exceeded a critical level. This critical protein concentration was at least about 8 g protein per 100 g of water, equivalent to 7.4%.

**Gel Structure.** The effect of pH on gel structure was examined by transmission electron microscopy. Comparisons were made between gels produced at pH 6.4 and those produced at 7.0 (Figure 5). Gel structures formed at both pH 6.4 (a) and 7.0 (b) consisted mainly of a network of myofibrils, indicated in the figure by either single or double arrowheads. Single arrowheads point to longitudinal sections of the myofibrils from which A-band and I-band are clearly seen. Double arrowheads point to transverse sections of myofibrils. The majority of the myofibrils were sectioned at different angles due to their random orientation in the gel structure. There are numerous empty spaces in the micrograph which can be put into two categories: one is the intramyofibrillar space and the other is the extramyofibrillar space. The spaces are the sites of the capillary forces holding imbibed water. The intramyofibrillar space is located inside the myofibrillar structure, and the extramyofibrillar space is located outside of the myofibrils. The intramyofibrillar spaces are much smaller than the extramyofibrillar spaces.

At neutral pH, an additional network of fine strands was formed. This network was intermixed with the main network of myofibrils. These fine strands were represented in the thin section micrograph by numerous small dots (arrow in Figure 5, panel b). These fine strands could contribute to gel strength. Their contribution to the water-holding capacity is also possible because of the fact that these fine strands could further divide extramyofibrillar spaces into smaller spaces. There appeared to be very few of these fine strands at pH 6.4 (Figure 5, panel a).

**Myofibrillar Structure.** Myofibrils embedded in the gel structure were further detailed by increasing the magnification. These myofibrils were sectioned longitudinally so that the myofibrillar structure could be seen clearly (Figure 6). The I-band (thin filaments) and A-band (thick filaments) are indicated by empty and solid arrows, respectively. After heating, once ordered, the parallel structure of the thin and thick filaments was disorganized at either pH 6.4 or 7.0. The intramyofibrillar spaces can be grouped into two basic categories according to their locations: spaces at I-bands and spaces at A-bands. The spaces at I-bands were larger than those at A-bands. The thin filaments showed a tendency to be condensed toward the thick filament region. At neutral pH, the thick filaments formed a porous network locally (Figure 6, panel b). These local networks of thick filaments tended to be separated along the M-line that is located in the middle of A-band. The thin filaments (I-band) were largely broken and possibly attached to the local networks of the thick filaments next to them. At pH 6.4, the thick filaments also formed local networks; however, their structures were denser than those developed at neutral pH, leaving less open

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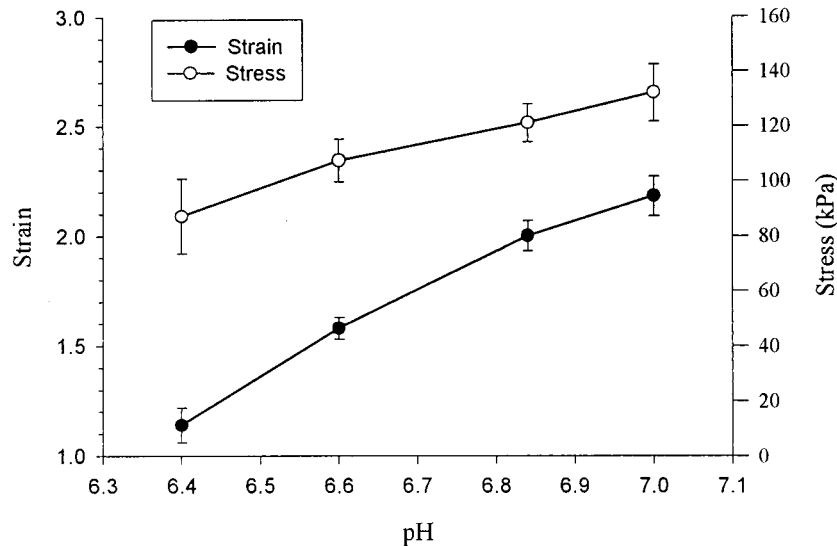


**Figure 2.** Effect of pH on stress and strain values at failure of heat-induced gels of twice water-washed chicken-breast muscle at 150 mM NaCl. Gel preparation: the sodium chloride concentration of the twice water-washed, minced chicken-breast muscle was adjusted to 150 mM, followed by adding 4% sorbitol and 4% sucrose. The pH of the final paste was adjusted to 6.4, 6.6, 6.8, and 7.0 in four separate samples. The pastes were cooked at 72 °C for 30 min. Strain and stress values were measured after the gels were stored for 24 h at 5 °C. The moisture contents at pH 6.4, 6.6, 6.8, and 7.0 were 70.4, 71.0, 71.0, and 72.0%, respectively. Vertical bars represent standard deviations.

**Table 1. Effect of pH on the Water-Holding Capacity of Heat-Induced Gels of Twice Water-Washed, Minced Chicken-Breast Muscle at 0.87% NaCl**

| pH final paste | moisture content (%) | | water loss (% of the total water in the final phase) | | | moisture content in final cooked, pressed gel[c] |
|---|---|---|---|---|---|---|
| | W2M2[a] | final paste | cooking loss | pressing loss | total water[b] loss | |
| 6.4 | 78.2 ± 0.4 | 70.7 ± 0.7 | 3.1 ± 0.1 | 40.6 ± 1.2 | 43.7 | 57.6 |
| 6.6 | 78.2 ± 0.4 | 71.4 ± 0.2 | 2.4 ± 0.2 | 30.8 ± 0.9 | 33.2 | 62.5 |
| 6.8 | 78.2 ± 0.4 | 71.5 ± 0.5 | 1.7 ± 0.1 | 15.5 ± 1.5 | 17.2 | 67.5 |
| 7.0 | 78.2 ± 0.4 | 72.0 ± 0.3 | 1.7 ± 0.1 | 13.4 ± 1.3 | 15.1 | 68.6 |

[a] Twice water-washed minced muscle. [b] The sum of the mean values of cooking loss and pressing loss. [c] The calculation is based on the initial moisture content of the final paste and the total water loss after cooking and pressing. Gel preparation is described in Figure 2.

space within the filaments to retain water. The thin filaments were largely kept in the I-band region, although they were apparently under strain because of the condensation of the thick filaments toward the M-line.
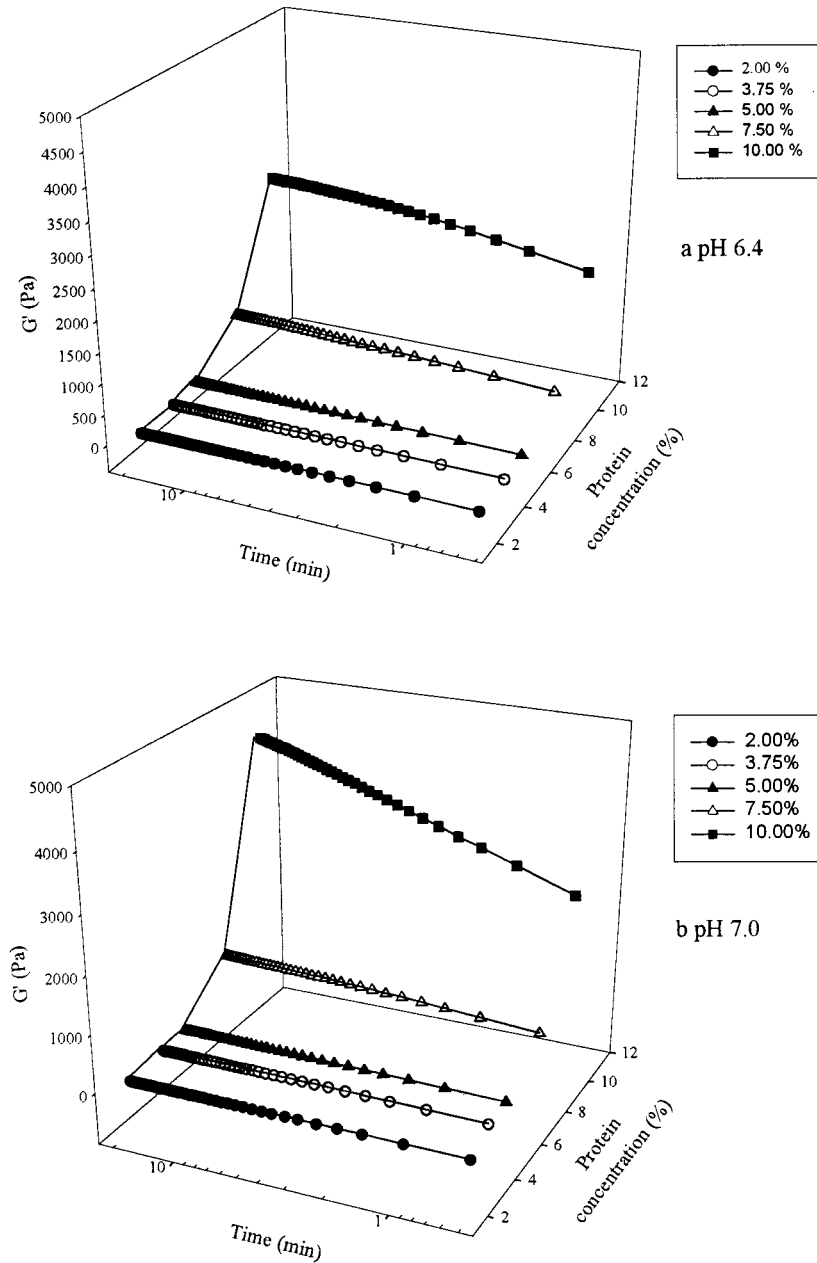
DISCUSSION

Addition of salt in the amount of 2–3% (427–641 mM sodium chloride, calculated on a basis of 80% water in the tissue) is the common practice in making gels from muscle proteins in the food industry. This amount of salt triggers the partial extraction of salt-soluble myofibrillar proteins, including myosin (*26*). Traditionally, solubilization of myosin is thought to be responsible for gelation because it is the main component of the thick sol that acts to bind together the insoluble components (*27*). It is theorized that the salt-soluble proteins partially unfold during heating and then reaggregate on further heating into a cross-linked, three-dimensional network (*28*). On the contrary, recent studies have found that comparable gels can be formed at reduced salt concentrations, e.g., 150 mM sodium chloride, where myosin molecules, along with a great majority of the other myofibrillar proteins, are not solubilized (*11*). However, the gelling ability depends strongly on the pH. A neutral or slightly higher pH is required. When gels are formed at high salt concentration, a slightly acidic pH is often adapted (*29*).

The analysis of microstructure has been widely used to visualize the gel structure and the influence upon it

of processing variables (*19*). The micrograph in Figure 5 showed that neutral pH facilitated the formation of a more evenly distributed or homogeneous gel matrix than was formed at pH 6.4. Sato et al. (*30*) reported that there is a direct relationship between the dispersion of the network components and elasticity. Elastic gels are derived from those having homogeneous structures in which the number of cross-links is high. Poor gels are from those with an unevenly distributed network where many of the myofibrils are aggregated locally as we observed here (Figure 5, a). The connections between these localized aggregates are expected to be weak within the gel structure. When a stress or strain is applied, the gel structure could be easily broken at these weak points.

Although it is not quite clear as to how neutral pH facilitates the formation of an evenly distributed network of myofibrils, several factors might be worth considering. pH adjustment from 6.4 to neutrality increases the net negative charge of the myofibrillar proteins. An increased electrostatic repulsive force could help to prevent myofibrils or myofilaments from forming localized aggregates. Stading and Hermansson (*31, 32*) found that β-lactoglobulin formed a particulate network structure when the electrostatic repulsion between protein molecules was weak but a fine-stranded structure when protein–protein repulsion was strong. Considerable swelling of the myofibrillar structures was observed when the pH was adjusted to 7.0 from 6.4 (Figure 5). Consistency or apparent viscosity increases

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**Figure 3.** Effect of protein concentration and pH (a, pH 6.4; b, pH 7.0) on storage modulus development of twice water-washed, minced chicken-breast muscle at 150 mM NaCl.

linearly with the volume fraction of particles (*33*). Increase in viscosity could reduce the mobility of the myofibrils during the heating process which could reduce the amount of aggregation. In addition, increasing the pH from 6.4 to 7.0 at 150 mM NaCl could increase viscosity because it solubilizes more of the myofibrillar and cytoskeletal proteins (*22*). There was approximately a 50% increase in the amount of proteins solubilized from minced, washed chicken-breast muscles when the pH was raised to neutrality. Most of these proteins that were additionally solubilized were of considerable size and could be expected to increase the apparent viscosity of the mixture. These extra proteins released at pH 7.0, compared to those released at pH 6.4, are proteins related to maintenance of the structure of the Z-disks and intact thick filaments. On solubilization, i.e., removal of these proteins from their structural elements, it would be expected that a further swelling of the myofibrillar structures could occur.
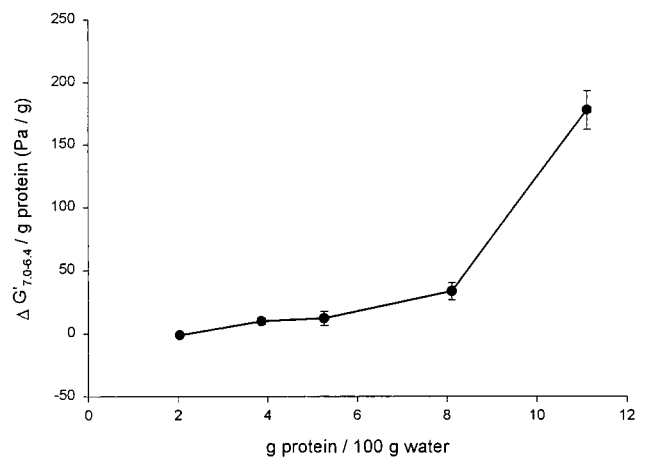


**Figure 4.** Effect of protein concentration and pH on storage modulus development at 150 mM NaCl.
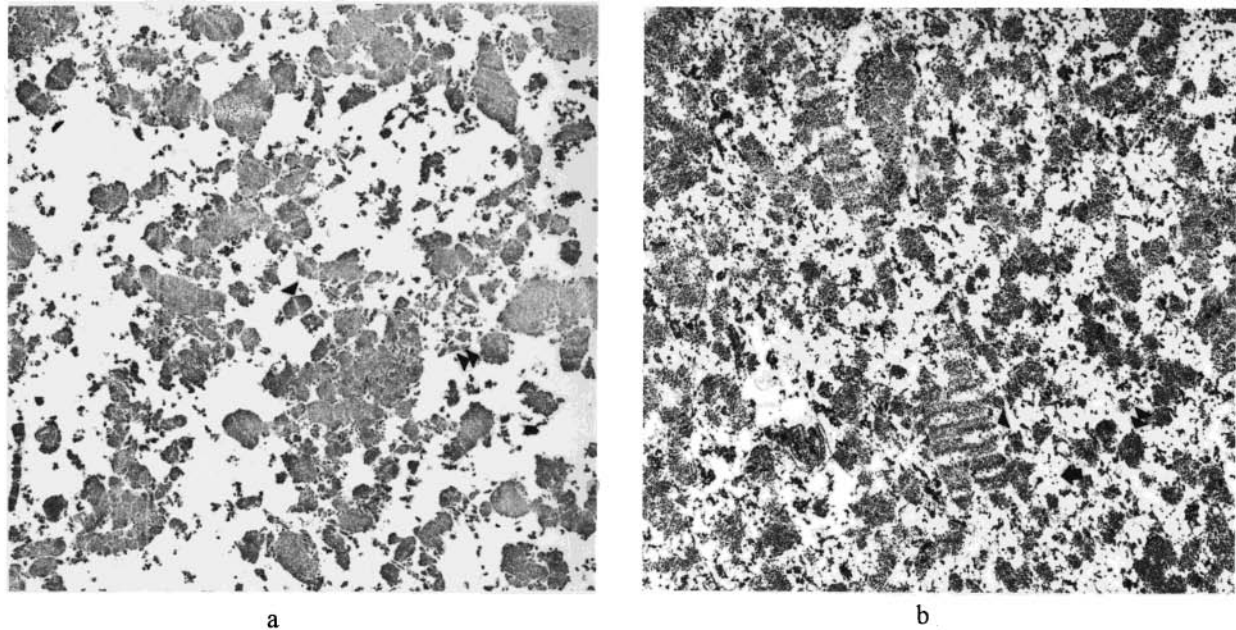
**Figure 5.** Effect of pH on the gel structure of heated, twice water-washed, minced chicken-breast muscle at 150 mM NaCl (a, pH 6.4; b, pH 7.0). The moisture content of the final paste was adjusted to 81.0% in both samples at pH 6.4 and 7.0. The paste was stuffed into a metal tube with a diameter of 10 mm and cooked at 72 °C for 30 min. Images were viewed under a transmission electron microscope. The image magnification is 4200× (arrow, fine strands; arrowheads, longitudinal sections of myofibrils; double arrowheads, cross sections of myofibrils).

Indeed, it is thought that removal of these "binding" proteins is necessary for the increased repulsive forces brought on by the increase in net negative charge to be effective in dispersing the insoluble myofibrillar proteins.

It was noticed that the paste developed at neutral pH before heating was stiffer than that which developed at pH 6.4. Chicken-breast muscle proteins undergo more extensive unfolding at lower temperatures at neutral pH than at pH 6.4 (*34*). It has been proposed that unfolding exposes more hydrophobic sites on the proteins and subsequent protein interactions stabilize the gel (*35*). Thus, carrying out the heating process for gelation at neutral pH might allow the sol-to-gel transition to occur at a lower temperature, which would aid in stabilizing the gel structure that is initially formed.

Niwa et al. (*36*) reported that surimi gels (high salt) usually exhibit the properties of an entropy–elastic body. The entropy of the gel structure is reduced under depression and the elasticity of the gel is expressed by its tendency to restore its entropy. Adjustment of pH to neutrality before heating would theoretically increase the entropy of the system by extracting and disorganizing structural proteins as described above. The swelling of myofibrillar structures that occurs at neutral pH would allow protein molecules a greater degree of freedom than they had before swelling. Depression of a gel formed at pH 7 would be expected to decrease the entropy of the system more than would occur for a gel formed at pH 6.4 because of the greater randomness of the proteins in the former case. Thus, there would be a greater driving force to restore the original randomness of the pH 7 gel compared to that at the lower pH which would increase the elasticity of the gel at the higher pH.

There appeared to be a large number of fine strands in the heated gels at pH 7 which were present to only a limited extent at pH 6.4. Formation of a network of fine strands at neutral pH could enhance the gel strength and impose restrictions on the reorientation of myo-

fibrils during heating. These fine strands could have been formed in part from the proteins solubilized from chicken-breast muscle at neutral pH and physiological ionic strength (*22*). These proteins have been tentatively identified as X-protein, C-protein, α-actinin, and desmin. The total amount of protein extracted with and without pH adjustment to neutrality was about 31 and 20%, respectively. The extra 11% of proteins consisted of those that were poorly extracted at pH 6.4. Solubilization of these proteins was correlated with the subsequent solubility of the myosin of the minced muscle in water. It is not possible to say what role these filaments might play in gelation, what proteins are actually involved in their formation, or how the heating process contributes to their formation.

Because myofibrils are the main components of the gel structure, the water-holding capacity of each myofibril is vital to the overall water-holding capacity of the gel. A larger intramyofibrillar volume should retain more water. Neutral pH facilitated the swelling of the myofibrils compared to a slightly acidic pH (Figure 6). Swelling of myofibrils is due to an expanded interfilamental spacing (*37, 38*). Adjustment of pH to neutrality from an acidic pH increases electrostatic repulsive forces and removes structural proteins that inhibit myofibrils from swelling (*22, 34, 39*). During heating, myofibrillar proteins denature and the muscle tissue shrinks. The increased electrostatic repulsive forces are helpful in inhibiting excessive aggregation of the proteins and shrinkage of thick and thin filaments. It is believed that the electrostatic repulsion developed after pH adjustment to neutrality is the key to ensure an increased water-holding capacity of myofibrils in the gel structure at physiological ionic strengths.

To improve the water-holding capacity of the gel structure, it is essential to improve the water-holding capacity in the extramyofibrillar spaces. Water is usually released from the intramyofibrillar spaces upon cooking. Water in extramyofibrillar spaces is largely

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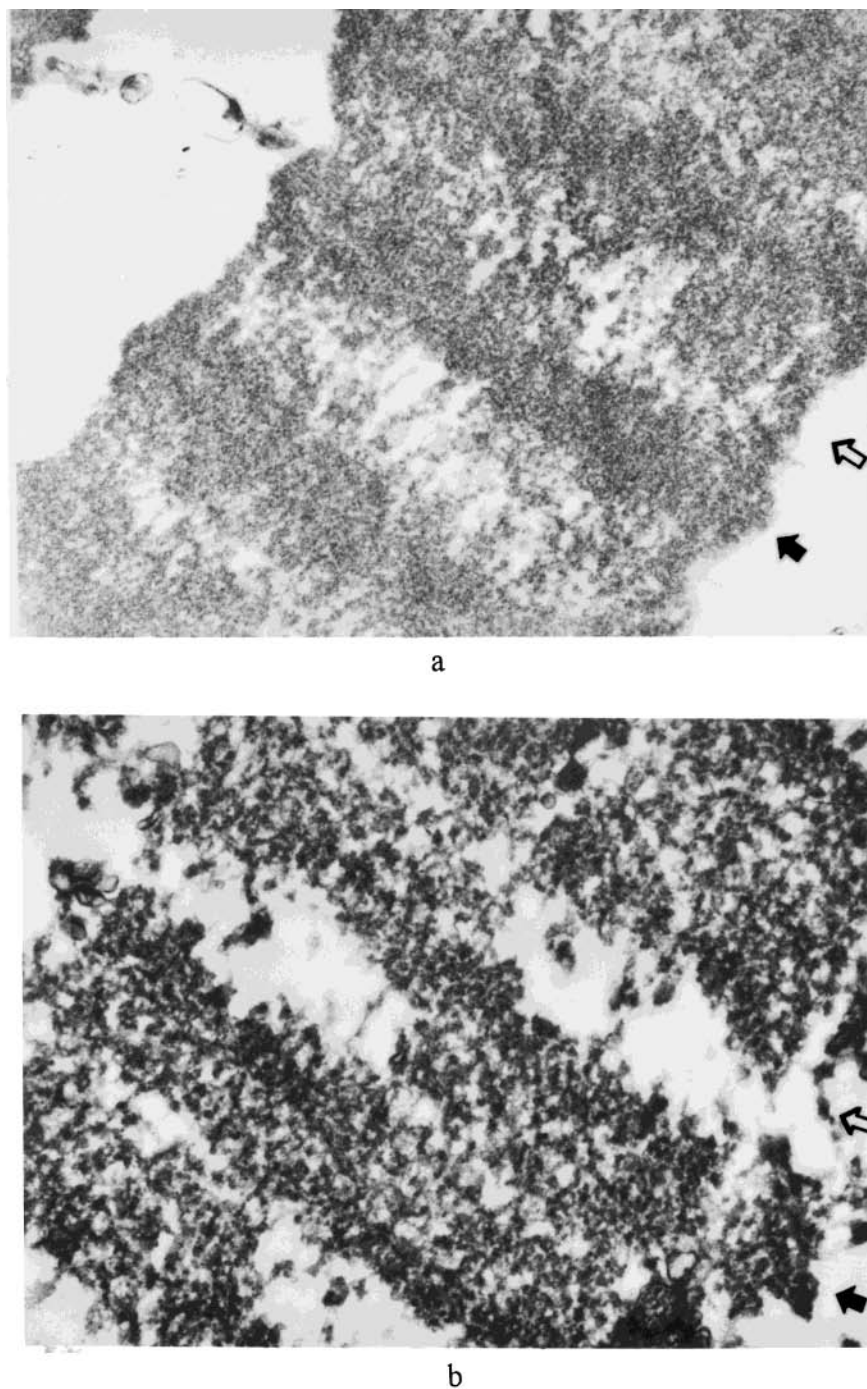


**Figure 6.** Effect of pH on the myofibrillar structure of heat-induced gels of twice water-washed, minced chicken-breast muscle at 150 mM NaCl (a, pH 6.4; b, pH 7.0). Images were viewed under a transmission electron microscope. The image magnification is 42000× (solid arrow, A-band; empty arrow, I-band).

immobilized by capillary force in the extramyofibrillar spaces whose diameters are inversely proportional to that force (*40*). The size and distribution of extramyofibrillar spaces should be a good indicator for the water-holding capacity under a set of defined experimental conditions. To retain as tightly as possible this extramyofibrillar water, it is desirable to form an evenly distributed network of myofibrils and a network of fine strands as was observed at neutral pH (Figure 5, b). The former narrows the variation of the pore size distribution and the latter reduces average pore size. At pH 6.4, large voids were created in which water could be easily pressed out when pressure was applied (Table 1, Figure 5, a). These large voids were surrounded by

large localized aggregates whose connections between each other were structurally weak (Figure 5, a). When a slice of gel is pressed, the gel structure collapses easily at these weak points and results in the extensive loss of water.

The optimum pH for the gelation of synthetic filaments at physiological ionic strength was reported to be around 6.0 instead of neutrality (*6, 14, 41*). Synthetic filaments were prepared by solubilizing myosin in buffer containing high salt concentrations, followed by lowering the ionic strength through dilution or dialysis. Researchers found that the rheological properties of myosin gels depended on the size of the reconstituted myosin filaments prior to the heating; the longer and

thicker filaments yielded firmer gels on heating (*6, 14, 15, 42*). One of the reasons for using a slightly acidic pH in these systems is that these synthetic filaments tend to dissociate at pH values greater than 6.5 (*15*). This explanation is, however, not applicable to myo-fibrils. Myofibrils are retained as a whole unit at neutral pH with some degree of swelling and disorganization (*34*). The swelling of myofibrils is beneficial for gelation and water-holding capacity because it increases the volume fraction of the dispersed phase (myofibrils) and improves the water-holding capacity in each myofibril. The gelling properties of synthetic filaments at physi-ological ionic strength have been investigated at a low protein concentration, in the range of 1−2%. It should not be expected that gels formed at high protein concentrations would be governed by the same mecha-nisms as those formed at low protein concentrations. The space-filling abilities of highly asymmetric and large molecules such as myosin, nebulin, and titin are greatly restricted, and their ability to become randomly distributed is limited at high protein concentrations (*43*). Thus, the supramolecular arrangement of these molecules at concentrations greater than 10% could be very different than that at the low concentrations discussed above.

## ACKNOWLEDGMENT

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