Role of Reduced Ionic Strength and Low pH in Gelation of Chicken Breast Muscle Protein

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ABSTRACT: Elastic gels with a high moisture content of 88% were prepared at an acidic pH and low ionic strength. The relationship among pH, ionic strength, water-holding capacity, and fold score of gels was investigated. A decrease of pH from 4.1 to 3.7 or below increased gel elasticity and significantly decreased water loss under pressure ($P < 0.05$). In the presence of sodium chloride, gels made at pH 3.5 to 3.7 had decreased elasticity and increased water loss under pressure. Prior freezing increased the water loss of gels under pressure. Gels made with phosphoric acid and hydrochloric acid lost less water under pressure than those made with citric acid. The percentage loss of water from cylindrical gels was inversely related to the height of the cylinders, suggesting that surface effects were involved. These results suggest that net positive charges on the protein molecules at low pH produced electrostatic repulsion, which was a major driving force for water uptake in the low-salt gels.

Keywords: muscle, chicken breast, protein gelation, ionic strength, low pH, water-holding capacity, water loss, gelation

Introduction

The generally accepted hypothesis to explain the formation of fish gels has been that a high concentration of NaCl (2% to 3%) is required to solubilize the myofibrillar proteins (Lee 1984,1986; Sano and others 1990). The optimum pH for gelation is neutral or slightly acidic. During heating of surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds. When sufficient bonding occurs, a 3-dimensional network is formed, resulting in gel formation. Four main types of chemical bonds can link proteins: hydrogen bonds, ionic linkages, hydrophobic interactions, and covalent bonds (Lanier 2000).

Recent studies in our laboratory have suggested that electrostatic repulsion plays an important role in protein gelation at physiological ionic strength, and strong gels can be formed at low ionic strength where myofibrillar proteins are not soluble (Chang and others 2001a, 2001b; Feng and Hultin 2001). Strain values in chicken breast muscle protein gels made at 0.15% salt at pH 7.0 to 7.4 and pH 6.0 to 6.5 were 1.9 and 0.7, respectively; the adjustment of pH from acidic to neutral significantly improved gel strength (Chang and others 2001a). Images from phase contrast microscopy and transmission electron microscopy showed that at pH 6.4 and physiological ionic strength, myofibrils formed a network of localized aggregates leaving large voids between, whereas at neutral pH, myofibrils formed a more evenly distributed network (Feng and Hultin 2001). The adjustment of pH from 6.4 to 7.0 had a dramatic effect on water uptake and water-holding capacity of gels made at physiological ionic strength. Without any added NaCl, an increase in gel pH (pH 6.4 to 7.0) also led to dramatic increases in water-holding capacity and water uptake (Kristinsson and Hultin 2003a).

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A neutral or slightly acidic pH is often chosen for making fish protein gels. A few articles have reported protein gelation under acidic condition (Fretheim 1985;Venugopal 1994; Venugopal and others 1994; Lian and others 2002). Fretheim and others (1985) found that myosin solutions (10 mg/mL) formed gels at 5 °C if the pH was decreased slowly by dialysis in the region of 5.5 to 2.5. Concentrations of 0.2 to 0.6 *M* salt (KCl) were found to increase gel strength linearly. Washed, collagen-free shark muscle protein formed gels when the pH was lowered to 4.5 by the weak organic acids, acetic or lactic acid, but strong acids, such as HCl, did not induce gelation (Venugopal and others 1994). The addition of NaCl, KCl, and $CaCl_2$ at 20 m*M* or less inhibited the low pH–induced gelation of shark myofibrillar proteins. Alaska pollock surimi formed strong gels at a pH of 4 with the addition of 2% to 3% acetic acid (Lian and others 2002). Gels formed at pH 3.92 had significantly higher firmness, less shrinkage, and stronger water-holding capacity than gels formed at pH 4.8. The addition of 1.5% NaCl or KCl decreased the firmness of the acetic acid–induced gels.

The objective of this research was to study the gelation of chicken breast muscle protein at acidic pHs and low ionic strengths to evaluate a possible role of electrostatic repulsion force on gelation. These repulsive forces would be derived from the net positive charge on the proteins, most of which have isoelectric points of approximately pH 5.0 to 5.5. Gel strength and water loss with heating and under pressure as a function of pH and ionic strength were measured.

Materials and Methods

Preparation of water-washed chicken breast muscle protein

Chicken breast muscle (Purdue brand, Perdue braqnd, Perdue Foodservice, Horshan, Pa., was bought in a local supermarket and transported to the laboratory on ice. Thirty grams of ground muscle was homogenized in 300 mL of cold, distilled, deionized water in a commercial Waring Blendor (Waring Products Div., Dynamics Corp. of America, New Hartford, Conn., U.S.A.) for 1 min. The ho-

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mogenized slurry was filtered through 4 layers of cheesecloth to remove unhomogenized muscle residue and connective tissues, and the filtrate was centrifuged in a Sorvall Centrifuge (Sorvall Seperspeed RC2-B Centrifuge, Dupont Medical Products, Wilmington, Del., U.S.A.) at 10000 × *g* for 20 min at 4 °C. The supernatant was decanted and the sediment was washed again with 300 mL of distilled, deionized water and centrifuged twice more, as described previously. This material will be called the "water-washed chicken breast muscle protein."

Gel preparation and cooking

The water-washed chicken breast muscle protein was adjusted to approximately 88% moisture content except where indicated. Protein gels were made by adjusting the pH to acidic values with 1 *N* HCl and chopped in a food processor (Sunbeam Oskar Household Food Processor, Sunbeam Applicance Co., France). In some cases, 0.5 *M* citric acid or 1.02 *M* (10%) phosphoric acid was used to adjust the pH. In some samples, NaCl was also included. The processor had been previously chilled in the refrigerator for several minutes. All chopping was done in the refrigerator, and the temperature of the paste was kept below 12 °C at all times. The samples were then stuffed into cellulose casings (21-mm dia) and cooked in a water bath at 90 °C for 20 min. At the end of this time, the casings were transferred into an ice bath for 30 min. Gels were removed from the casings and held at 5 °C in sealed plastic bags overnight until testing.

Water retention of gels

Water loss upon cooking. Cooked gels were analyzed for moisture content. From the water content after cooking and the initial water content of the gel paste, the water loss was calculated. Percent water loss upon cooking was calculated on the basis of the original water content of the gel paste.

Water loss of cooked gels under pressure. Cooked protein gels were cut into 3-mm thick and 21-mm dia slices and sandwiched between 5 layers of filter paper (P5, medium porosity, Fisher Scientific, Pittsburgh, Pa., U.S.A.). A flat plate was placed on the top of the sandwiched gels, and a 3-kg weight was put on the top of the plate to press the gel slice for 1 min. The weights of gel slices before and after pressing were taken. Water loss under pressure was determined by measuring weight change before and after pressing and is reported based on gel water content before pressing.

Water loss of cooked gels during cold storage. Cooked gels with a 21-mm dia were cut into 3-mm, 10-mm, and 25-mm high discs. Gel discs were put into a polyethylene box vertically and stored at 5 °C. Weights of gels during the whole storage period were followed. Water loss of cooked gels during the storage was calculated from the difference in gel water content during storage and is reported based on the starting water content in the gel.

Freezing/thawing of gels

Gels were cut into 25-mm-long cylinders, vacuum-packaged in polyethylene bags, and frozen at –38 °C for 1 wk. Frozen gels were thawed in the refrigerator for 1 d at 5 °C. Water loss of frozen gels on thawing and water loss of frozen/thawed gels on pressing were determined.

Gel elasticity

Fold test. The fold test according to Kudo and others (1973) was used to assess the elasticity of the gels. Gels, cut into 3-mm-thick slices, were subjected to a double fold and given scores according to their resistance to crack. The fold score was 5 if no crack occurred after folding twice, 4 if no crack occurred after folding once, 3 if the slice cracked gradually when folded in half, 2 if the slice cracked immediately when folded in half, and 1 if the slice broke under finger pressure.

Punch test. The punch test was conducted with a TA-XT2 texture analyzer (Texture Technologies, Corp., 6 Patton Drive, Hamilton, Mass., U.S.A.). Gels were cut into a cylinder of 25-mm height and 21-mm dia. A spherical probe of 5-mm dia was used to measure resistance and depth before breaking. TA-XT2 settings were as follows: mode, measure force in compression; option, return to start; pretest speed, 1.0 mm/s; test speed, 1.1 mm/s; post-test speed, 10.0 mm/s; distance, 15 to 18 mm; trigger force, auto-10 g; data acquisition rate, 200 pps. The force to puncture the gel (breaking force) and the distance at which the ball probe penetrated the gel before breaking (breaking distance) were recorded. Gel strength was measured according to 3 parameters, maximum force (breaking force), distance to rupture (breaking distance), and "gel strength." Gel strength is the peak force in grams multiplied by the distance to the rupture event measured in centimeters.

Solubility measurement. The solubility of the water-washed chicken breast muscle protein was determined according to the method of Krishnamurthy and others (1996). Four grams of muscle protein was homogenized in 200 mL of distilled, deionized water for 1 min, and the pH was adjusted with 0.5 *N* HCl; the suspension was centrifuged at 18000 × *g* for 20 min at 4 °C. Samples were taken for protein determination before and after centrifugation. Percent solubility was calculated by taking the ratio of the protein in the solution after centrifugation divided by the total protein in the suspension before centrifugation. Protein content was measured by the Lowry procedure (Lowry and others 1951) or by the Biuret method (Layne 1957).

Statistical analyses. Data were analyzed by analysis of variance (ANOVA) using the General Linear Model procedure of SAS 8.2 (SAS institute Inc., Cary, N.C., U.S.A.). Differences between treatment means at the 5% level were determined using the Duncan New Multiple Range Test (DNMR). Regression analysis was conducted using Microsoft Excel XP (Microsoft Corp., Redmond, Wash., U.S.A.).

Results and Discussion

Gels prepared at different pHs

The equivalent ionic strength in the minced water-washed chicken breast muscle was less than 1 m*M*. Gels made with the minced water-washed chicken breast muscle with a moisture content of 87.2% at acidic pH and without the addition of sodium chloride are shown in Table 1. Gels formed at pH 3.16 were superior to those at pH 3.70 or 4.06. Gels could not be formed at a moisture content of 87.2% with 2.5% NaCl at a neutral pH (data not shown).

The sample at pH 4.06 lost 12.1% of its water on cooking, whereas essentially no water was lost at the lower pH values. Likewise, the percent water loss that was obtained on pressing was more than 3 times greater at pH 4.06 than at pH 3.70, 3.40, or 3.16. This was in spite of the fact that the cooked gels at pH 4.06 contained less water than those at the lower pH (Figure 1). The percent water loss on pressing between pH 3.70 and 3.16 was not significantly different ($P < 0.05$).

Gels made at different ionic strengths

Water loss of gels during cooking and under pressure and fold scores of cooked gels were determined with 0 to 50 m*M* added NaCl. The pH in the pastes was between 3.50 and 3.65 in all samples, and the moisture contents in the initial pastes varied from 87.5% to 88.4%. There was little or no water loss when cooking at 90 °C for 20 min at any level of NaCl (Table 2). Further addition of

Table 1—Characteristics of chicken breast muscle protein gels made with HCl at acidic pHs[a]

pH of paste	Moisture content of cooked gel (%)	Force[b] (g)	Deform[b] (mm)	Gel strength (g * cm)	Fold score[c]
4.06	85.8	209.5 ± 8.4	6.2 ± 0.12	130.8 ± 2.7	2
3.7	87.2	227.4 ± 7.4	5.6 ± 0.18	128.1 ± 6.7	3
3.4	87.7	ND	ND	ND	3.5
3.16	87.6	260.2 ± 11.2	7.4 ± 0.25	192.2 ± 1.8	3.5

[a]ND = not determined.
[b]Three duplicates were determined.
[c]Score scale is from 1 to 5; 2 represents a gel structure that cracked immediately when folded in half, 3 represents a gel structure that cracked gradually when folded in half, and 4 represents a gel structure that no crack occurred after folding once.

salt into the gels decreased the fold scores. Fold scores of 5 were achieved for gels with 0 or 5 m*M* salt addition. Higher fold scores (5) were obtained in the gels prepared without any added salt (Table 2) than were obtained under similar conditions in the experiments reported in Table 1 (3.5). There were many uncontrolled factors in these experiments, among which would be the state of the raw material and chopping process in gel preparation. That is why each factor evaluated had an internal control. The chicken breast muscle samples used in Table 1 and 2 were from 2 different batches. Thus, it is not possible to make direct comparisons between these 2 sets of experiments. There were significant differences in water loss of the cooked gels under applied pressure as a function of NaCl addition ($P < 0.05$) (Table 3). The losses were relatively low up to 20 m*M* NaCl but increased substantially with the addition of 50 m*M* salt. The gels with no salt addition had the lowest loss of water (10.3%) under the pressure applied.

Cooked gels were placed in storage at –38 °C for 1 wk and thawed at refrigerator temperature overnight. The freeze-thaw process caused a relatively small release of water from the gels, with the gels with the 2 lower contents of added salt losing less water than the others (Table 3). The water losses of frozen gels with 0 to 50 m*M* added NaCl on thawing were not significantly different ($P < 0.05$). A much greater amount of water was lost under pressure compared with that lost by freezing/thawing. There was no significant difference ($P < 0.05$) in water loss under pressure as a function of salt concentration, except for the highest salt content (50 m*M*), where a greater amount of water was released. Water loss from the frozen/thawed gels was greater at all salt concentrations than water loss from the unfrozen samples under the pressure applied.

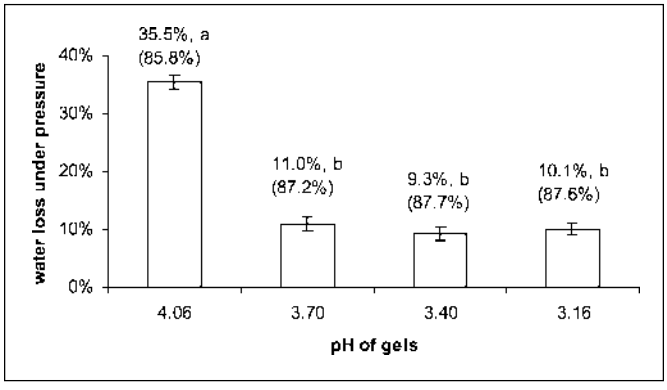

Figure 1—Water loss of chicken breast muscle protein gels made with HCl under pressure at different pHs. The data on the top of columns were actual water loss of chicken breast muscle protein gels under pressure, and the data in parentheses were moisture contents of gels before pressing. Means with different letters on the top of columns are significantly different ($P < 0.05$). Water loss under pressure was calculated from the difference in gel water content before and after pressing and is reported based on gel water content before pressing.

Table 2—Characteristics of chicken breast muscle protein gels made with HCl at different ionic strengths[a]

NaCl added	pH in final pastes	Moisture content in pastes (%)	Moisture content in gels (%)	Fold score[b]
0 m*M*	3.58	88.4	88.2	5
5 m*M*	3.55	88.0	88.0	5
10 m*M*	3.65	88.1	88.1	3.5
20 m*M*	3.50	87.6	87.5	2.5
50 m*M*	3.53	87.5	87.5	2

[a]Gel preparation and details of the double fold test are described in the "Materials and Methods" section.
[b]Score scale is from 1 to 5; 2 represents a gel structure that cracked immediately when folded in half, 3 represents a gel structure that cracked gradually when folded in half, and 5 represents a gel structure that can pass double fold test without breakage.

Solubility of water-washed chicken breast muscle protein

In regular surimi gels, 2% to 3% of salt is added for "solubilizing" myofibrillar proteins and subsequent gelation (Lee 1984, 1986). Water-washed chicken breast muscle protein was suspended in 50 volumes of distilled, deionized water, and its solubility over a pH range of 3.05 to 7.5 was determined. Water-washed chicken breast muscle protein was least soluble around its isoelectric point pH (5.5). As the pH shifted from this value in either direction, protein solubility increased (Figure 2). Solubility at pH 4.45 was 5.2% and increased sharply to 86.3% at pH 3.78. Below pH 3.78, solubility increased further. At pH 7.5, protein solubility was 22.6%.

Gels made with different acids

Two inorganic acids, hydrochloric and phosphoric, and 1 organic acid, citric acid, were used to prepare acid-induced gels. There was little water loss during cooking with any of these acids (data not shown). When the cooked gels were pressed, the water loss in the gels made from citric acid (28.7%) was over twice that from phosphoric (11.5%) or hydrochloric (10.1%) acid (Figure 3). This was in spite of the fact that the initial water content of the citric acid gels had the lowest moisture content before pressing. The pH of the citric acid gels was slightly lower than the others. We have no explanation for this.

Water loss from gels of different heights

Gels of 21-mm dia made with HCl were cut into slices 3 mm, 10 mm, and 25 mm thick and stored at 5 °C to study water loss of cooked gels during cold storage. There was a continuous loss of water of the cooked gels during cold storage (Figure 4). After stor-

Table 3—Water loss during thawing and pressing of unfrozen and frozen/thawed gels[a,b]

NaCl added (m*M*)	Water loss of unfrozen gels under pressure[c] (%)	Water loss of frozen gels on thawing[d] (%)	Water loss of frozen/ thawed gels on pressing[e] (%)
0	10.3 ± 0.5c (87.7)	1.98 ± 0.37a (88.2)	30.4 ± 2.2b (88.0)
5	13.0 ± 0.9b (87.4)	3.12 ± 0.66a (88.0)	32.3 ± 5.5b (87.7)
10	12.3 ± 0.4bc (87.7)	3.76 ± 1.97a (88.1)	33.2 ± 3.6b (87.7)
20	13.5 ± 0.8b (86.5)	4.68 ± 1.86a (87.5)	29.6 ± 2.0b (87.0)
50	34.2 ± 3.0a (87.0)	3.83 ± 0.27a (87.5)	49.2 ± 0.8a (87.1)

[a]pHs of gels at different NaCl concentration are given in Table 2.
[b]Means with different letters in the same column are significantly different at a level of 5%.
[c]% water loss of unfrozen gels under pressure was calculated from the difference in gel weight before and after pressing and is based on the water content of the unfrozen gels. The data in parentheses are moisture contents of unfrozen gels before pressing.
[d]% water loss of frozen gels on thawing was calculated from the difference in gel weight before and after freezing and is based on the water content in gels before freezing. The data in parentheses are moisture contents of gels before freezing.
[e]% water loss of the frozen/thawed gels on pressing was calculated from the difference in gel water content before and after pressing and is based on the water content in thawed gels before pressing. The data in parentheses are calculated moisture contents of thawed gels before pressing.

age for 250 h, gels with heights of 3 mm, 10 mm, and 25 mm lost 35.4%, 13.5%, and 6.8% of their water, respectively. Slices with the smallest height lost the most water. The relationship between the water loss after storage for 250 h and the gel height was regressed as follows: % water loss = –0.1376 ln (ln = natural logarithm) (height of gel slice, mm) + 0.4891, with an R^2 of 0.95. The results possibly indicate that the water loss is a surface phenomenon as opposed to being driven primarily by hydrostatic pressure.

Discussion

The purpose of these experiments was to evaluate the gelation characteristics of chicken breast muscle formed at low pH and low ionic strength. The low ionic strength was achieved by washing the minced chicken breast muscle 3 times in 10 volume of water to reduce the amount of the native salts in the muscle tissue. It was shown that gels containing up to 88% moisture could be formed at pH values of 4.06 to 3.16, giving values of almost 200 g-cm and fold test scores of 5 when there was no salt added into the washed chicken breast muscle mince from which the gels were made. Gels could not be formed at pH 5.0 and higher under these same conditions.

The actual pH at which the gels were formed had an effect on the properties of the gels. Gels formed at pH 3.16 were superior to those formed at 3.70 and 4.06 with respect to force and deformation at puncture (Table 1), whereas water-holding capacity was better at a pH value of 3.70 to 3.16 compared with 4.06 (Figure 1). There was very little difference between water loss under pressure at pH 3.70 versus 3.16. Overall, the ability to form better gels was improved as the pH was lowered over this range.

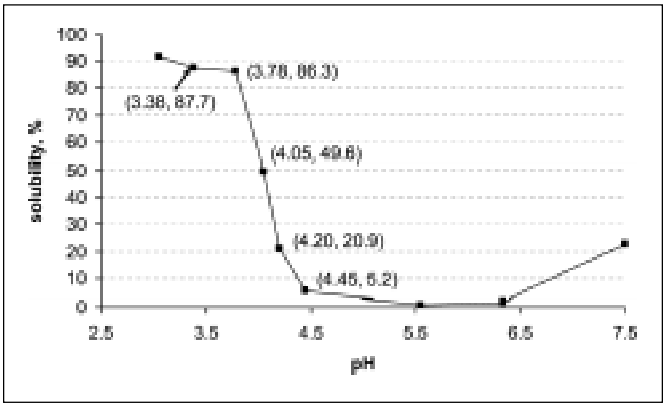

Figure 2—Solubility of twice-washed chicken breast protein at different pHs. The data in parentheses are pH and solubility, respectively, at the indicated points.

When gels were prepared at pH values of 3.50 to 3.65 at increasing concentrations of salt, there was very little loss in moisture when pastes containing 87.5% to 88.2% water were converted to gels by heating (Table 2). Elasticity of the gels was best when there was no added salt or only 5 m*M* salt as measured by fold score, and there was decreasing elasticity as the salt content increased to 50 m*M*. There was also an increase in water loss of the unfrozen gels when the salt concentration was increased to 50 m*M* compared with 20 m*M* or less (Table 3).

These results are consistent with the theory that the water-holding capacity of the gels is primarily a reflection of the electrostatic repulsion occurring between the proteins as they enter into a pH range in which the net positive charge becomes large enough to overcome the attractive forces between the protein molecules. It seems probable that the major change in the proteins in this pH range occurs through the removal of negative charges on the proteins by protonation of the carboxylate groups of aspartic and glutamic acid residues. These residues make up more than 25% of the amino acid residues in myosin (Bodwell and McClain 1978). The pKa's of the aspartic acid and glutamic acid residues are approximately 4.7 in some proteins (Tanford 1961; Lehninger 1970) but

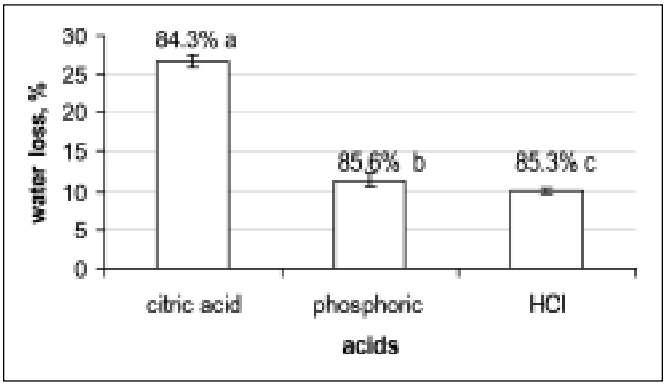

Figure 3—Water loss under pressure of chicken breast muscle protein gels prepared with different acids. The concentration of citric acid in the paste was 47 m*M*, and the pH of the paste was 3.58; the concentration of phosphoric acid in the paste was 38.4 m*M*, and the pH of the paste was 3.70; the concentration of HCl in the paste was 93.1 m*M*, and the pH of the paste was 3.73. Percent water loss is based on the water content of the cooked, unpressed gels. Data on the top of the column were moisture contents in the gels before pressing. Means with different letters on the top of columns are significantly different at a level of 5%.

may run as low as 3.65 for aspartic acid and 4.32 for glutamic acid (Hardy 1985). It seems reasonable to expect that protonation of the aspartic and glutamic acid side chains results in both formation of the gels as well as the development of the electrostatic repulsive force between the protein molecules. The protein gels form when the charge is reduced on the protein to a value that lowers electrostatic repulsion to the point that other forces such as hydrophobic bonds can exert their effect to aggregate the proteins. Thus, it would be expected that gels could form under conditions in which the electrostatic repulsive forces were not maximal. This is consistent with our data, which show gels forming at pH 4.06 but with some improvement in water-binding and elasticity continuing to develop as the pH is lowered to 3.70 and even to 3.16. Undoubtedly the change in the charges of the surface groups of the proteins would lead to conformational changes, which probably favor the exposure of hydrophobic groups and their interaction. Although volume exclusion effects would also play a role (Elliot and Hodson 1998), the strong dependence of the changes in the gels with pH indicates that electrostatic repulsion is the major force driving the water-holding ability of the proteins. The role of increasing levels of sodium chloride would be to shield the positive charges on the proteins by the chloride ions, thus reducing the electrostatic gel pressure and lowering the ability of the proteins to hold water in their gel structure.

A freeze/thaw process of the cooked gels lowered the ability of the gels to hold water when subjected to pressure compared with the gels that were not frozen. The greater release of water between the frozen/thawed gels and the unfrozen gels occurred at all salt concentrations. However, with both the frozen and unfrozen gels, there was a sharp increase in the loss of water-holding ability at 50 m*M* sodium chloride compared with 20 m*M* sodium chloride or less. During the freezing process, the acids would be concentrated in any unfrozen water, probably producing a lower pH than in the unfrozen state. This low pH could induce conformational changes in the proteins, which hindered their ability to maintain the integrated protein structure needed to maintain good structure and retain the electrostatic repulsive force between the charged protein molecules. Conformational changes in proteins could affect the aggregation of the proteins and cause structural changes that weaken the gels (de Groot and de Jongh 2003). Low pH has been shown to reduce the hydrodynamic volume of proteins (discussed in Kristinsson and Hultin 2003b). Changes in conformation that led to a decrease in protein volume could have important consequences for electrostatic repulsive forces because these forces are inversely dependent on the 6th power of the distance between the charges.

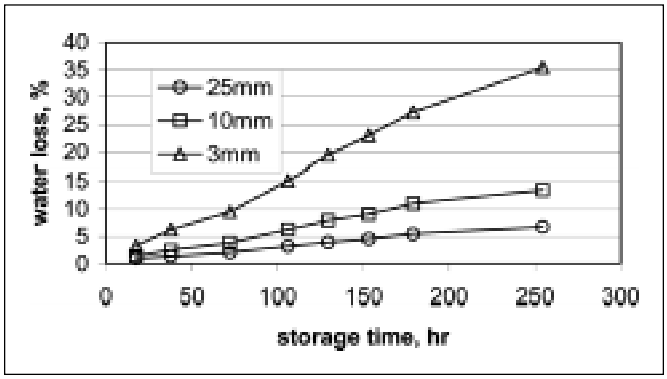

Figure 4—Effect of gel height on water loss during storage. Gel made with HCl as shown in Figure 3 was cut into 3-mm, 10-mm, and 25-mm high discs. Gel discs were put into a polyethylene box vertically. Water loss during storage at 5 °C was calculated from the difference in gel water content during storage and is reported based on the starting water content in the gel.

Gels could be formed at pH 4.06, and certain properties of the gels such as the ability to hold water under pressure became decidedly better at a pH of 3.7 or less. Although protein solubility is determined at low temperature in dilute suspensions and gels are formed at high temperatures at higher concentrations, it seems likely that it is more than a coincidence that this is the pH range over which the protein solubility was rapidly changing. At pH 4.05 we found that about 50% of the protein was soluble, whereas it went to 86% soluble at pH 3.78. The changes in the muscle proteins that may produce solubilization in the presence of sufficient water could lead to a disorganization of the muscle protein structures in more concentrated suspensions, which would allow the proteins in various cellular structures such as the thick filaments and the Z disks to be dispersed in the aqueous phase. Equal dispersion of protein should give the best gelation characteristics of any protein suspension (Feng and Hultin 2001). It is believed that the function of adding high salt to most muscle protein foods is to solubilize the proteins so they can be dispersed (Lee 1984, 1986). Thus, low pH has the same function as high salt concentrations in dispersing the protein.

It had previously been shown that neutral or slightly alkaline pH values can improve the gelation characteristics of chicken breast muscle gels, including their water-binding capacity (Chang and others 2001a, 2001b; Feng and Hultin 2001; Kristinsson and Hultin 2003a). Gel characteristics were improved at pH 6.8 or higher. Unlike the results obtained in this study at acidic pH, the conditions that gave improvement of gels on the alkaline side did not occur under conditions which, if carried out in the presence of adequate water, would lead to solubilization of most of the myofibrillar proteins. Only a few proteins had to be solubilized to accomplish the good results at neutral pH. This solubilization was achieved in a relatively low concentration of sodium chloride, that is, 25 to 150 m*M*. The proteins solubilized under these conditions appeared to come primarily from the Z disk and thick filaments (Krishnamurthy and others 1996). Removal of these proteins was believed to "untie" the proteins from the thick filaments, which then allowed the other proteins of the thick filaments to disperse. Dispersed proteins, whether or not under conditions in which they would become soluble in the presence of adequate water, form good gels. Thus, it is not primarily solubility but dispersion that is the important criterion.

At low pH it was not possible to see a separate effect of dispersion versus solubilization because of the close correlation between total protein solubility and gelation. The difference in the behavior of the proteins at low pH and at neutral and slightly alkaline pH is perhaps due to the high concentration of the carboxylate side chains in muscle proteins and the relatively narrow pH range over which the charge characteristics of these groups change. The side chain groups involved at the higher pH values are much fewer than the carboxylate side chains. Also, the range of pH of the pKa's of the side chains is much broader than at low pH. For example, whereas aspartic and glutamic acids side chains account for over 25% of the side chains of myosin, histidine with pKa values ranging from 6 to 6.8 constitutes only about 2% of the total residues (Bodwell and McClain 1978). Thus, changes in protein solubility are not so dramatic on the alkaline side where most of the side chains, for example, lysyl amino groups, have pKa's in the range of 9 to 10.2 (Tanford 1961; Hardy 1985).

Conclusions

The water holding capacity and gel strength of washed chicken muscle breast muscle protein gels without added salt were highly dependent on the gelation pH. At acid pH, elastic gels with water content as high as ~88% were formed. As the pH decreased from the isoelectric point of the major proteins (~55%), water holding capacity and gel strength increased. Adding salt up to 50 mM decreased the water holding capacity and gel strength of acid-induced gels significantly. Prior freezing of acid-induced gels also caused a greater water loss when subjected to the pressure than the unfrozen gels. These results are consistent with the theory that the water holding capacity of the gel is primarly a reflection of the electrostatic repulsion occurring between the protein molecules as they enter a pH range where the net positive charges become large enough to overcome the attractive forces between the protein molecules. As the pH shifted from the isoelectric point to the acid side, the net positive charges on the protein molecules increased resulting in the expansion (or swelling) of the filament lattice.

Acknowledgments

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