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FUNCTIONAL PROPERTIES AND MICROSTRUCTURE OF CHICKEN BREAST SALT SOLUBLE PROTEIN GELS AS INFLUENCED BY pH AND TEMPERATURE

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Abstract

The effects of different pH's (4.5, 5.5, 6.5 and 7.5) and temperatures (30-80 °C) on the functional properties and microstructure of chicken breast salt soluble protein (SSP) in 0.6 M NaCl were investigated. Protein solubility decreased from 98% at pH 6.5 to less than 1% at pH 4.3 and below. Salt soluble protein at pH 4.5 produced a discontinuous gel which could not be measured by back extrusion and had an expressible moisture above 80% at all heating temperatures. Aggregated globular microstructures were observed at pH 4.5 by scanning electron microscopy (SEM). Thin-sectioned images as seen by transmission electron microscopy (TEM) suggested the globules were composed of loosely packed long protein filaments approximately 20 nm in diameter. Gels at pH 5.5 had the highest viscosity index between 45-80 °C and thickest SSP strands as viewed by SEM and TEM, but expressible moisture approached 80% when they were heated to 70 °C or above. Gels at pH 6.5 and 7.5 exhibited similar expressible moisture (approximately 40%), viscosity index and a regular, continuous network of linear globular strands at 65 °C by SEM. Microstructures at pH 5.5, 6.5 and 7.5 changed with temperature, with maximum strand diameters observed at 65 °C. Changing pH altered SSP solubility which influenced gel texture, water retention and microstructure.

Key Words: Gelation, microstructure, salt soluble protein, chicken, pH, texture, temperature, functional properties.

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Introduction

Gelation of salt soluble proteins (SSP) in muscle systems plays an important role in stabilizing fat and water, as well as developing desired texture in processed meat products. The principal components in SSP are myosin and actin. In SSP systems, myosin contributes most to gel formation and functionality (Samejima *et al.*, 1969, 1982; Yasui *et al.*, 1980). Yasui *et al.* (1982) reported a myosin-to-actin molar ratio of 2.7:1 was necessary for maximum gel strength in 0.6 M KCl, 20 mM phosphate buffer, pH 6.0. At this ratio, 15-20% of total protein existed as an actomyosin complex and the remainder was free myosin.

Different muscle types and species have different gel properties. Ishioroshi *et al.* (1979) reported that rabbit myosin gels were optimally developed at temperatures between 60 °C and 70 °C at pH 6.5 in 0.6 M KCl. The pH for maximum gel rigidity in 0.6 M KCl was reported at pH 5.4 to 5.6 for chicken breast myosin (Asghar *et al.*, 1984; Morita *et al.*, 1987) and at pH 5.1 for chicken thigh myosin (Morita *et al.*, 1987). The highest myosin gel strength at constant pH was observed in 0.1-0.2 M KCl. White myosin from chicken (Asghar *et al.*, 1984; Morita *et al.*, 1987) and bovine muscle (Fretheim *et al.*, 1986) generally exhibited superior gel forming properties at pH 6.0 than red myosin. Processed meat products are not formulated to a pH; thus it is important to understand the properties of muscle proteins over a range of pH's.

Microstructures of muscle protein gels have been studied using both transmission (TEM) and scanning electron microscopy (SEM) (Yasui *et al.*, 1979; Smith and Brekke, 1985; Hermansson, 1986; Hermansson and Langton, 1988; Choe *et al.*, 1989, 1991). Hermansson *et al.* (1986) observed two types of myosin gel structures after heat treatment using SEM. Fine stranded gel structures were formed at pH 5.5 and 0.25 M KCl, whereas an aggregated gel structure was formed at pH 6 and 0.6 M KCl. Yamamoto *et al.* (1988) proposed that the type of gel structure was dependent on the length of unheated myosin filaments from red and white muscle fibers. Short myosin filaments formed less rigid, aggregated gels upon heating, while long filaments formed fine-

stranded networks with high rigidity.

Conventional chemical fixation techniques used to localize proteins through intra- and inter-molecular cross-linking may result in structural modification of protein molecules (Hayat, 1981). Chemically fixed samples may not truly represent the natural state of the gel, but a chemically modified artifact. Comparison of different preservation techniques can enhance interpretation of protein gel microstructures. An alternative to chemical fixation is cryofixation which almost instantaneously arrests molecular movement and preserves the spatial relationship among molecules and three-dimensional structure (Hayat, 1989). Structural preservation using cryofixation is dictated by freezing speed which determines ice crystal size and accompanying distortion of structure (Bohler, 1979). Ultra-rapid freezing withdrew heat (10^4-10^5 °C/sec) from an unfixed sample so that no ice crystal damage was visible with the electron microscope (Gilkey and Staehelin, 1986).

The purpose of the present study was to demonstrate the effect of pH and temperature on changes in microstructure of chicken breast SSP gels preserved by chemical fixation and ultra-rapid freezing using SEM and TEM. Associated changes in protein solubility, gel strength as measured by viscosity index and water-holding capacity were also investigated.

Materials and Methods

Isolation of chicken breast salt soluble proteins

The SSP were isolated from hand-deboned chicken breast muscle by extracting twice in low salt (0.1 M NaCl, 50 mM Na phosphate buffer, pH 6.5) buffer and once in high salt (0.6 M NaCl, 50 mM Na phosphate buffer, pH 6.5) buffer (Wang *et al.*, 1990). The final pellet was solubilized in 1/3 volume of 2.4 M NaCl, 0.2 M Na phosphate buffer of the desired pH, to obtain a protein solution in 0.6 M NaCl. Protein concentrations were determined by the AOAC (1984) 24.038-24.040 Kjeldahl method, and diluted to 30 mg/ml with 0.6 M NaCl, 50 mM Na phosphate buffer of the desired pH. Final pH's were adjusted with 0.1 M HCl or NaOH, if necessary. The final SSP solution was characterized as 1.3:1 myosin-to-actin weight ratio using SDS-PAGE as described by Wang *et al.* (1990).

Protein solubility

The pH of SSP solutions was adjusted between pH 4.0 and 6.0, held overnight, and centrifuged at 20,000 x g for 30 minutes. Protein concentration of the supernatant was measured using Biuret method (Gornall *et al.*, 1949). The SSP solubility was calculated by dividing the protein concentration of the supernatant by the original SSP solution concentration and multiplying by 100.

Treatments

Salt soluble protein gels were prepared at 4 pH's (4.5, 5.5, 6.5 and 7.5) in 0.6 M NaCl and nine final temperatures (30 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C and 80 °C) for back extrusion and expressible moisture. Conditions selected for evaluating gels by SEM and thin-sectioning were four different pH's (4.5, 5.5, 6.5 and 7.5) with three final heating temperatures (55 °C, 65 °C and 80 °C) for SEM and two final temperatures (55 °C and 80 °C) in 0.6 M NaCl for TEM. Gels evaluated using the freeze-fracture technique were prepared at four different pH's (4.5, 5.5, 6.5 and 7.5) in 0.6 M NaCl at a final heating temperature of 80 °C.

Gel preparation

Gels were prepared as described by Smith *et al.* (1988). Eight grams of each 30 mg/ml protein solution were pipetted into a 16 x 100 mm screw top test tube with an inner radius of 6.75 mm. The tubes were capped, heated at a constant rate of 1 °C/min in a water bath monitored by a Nelsprit Temperature Programmer (Model MTP-6, Nelsprit, Inc., Newington, NH) and immersed in ice-chilled water upon reaching the desired internal temperature as measured using a thermocouple thermometer inserted into one sample. Gels were prepared one day before testing and stored at 4 °C until used.

Gel properties measurement

An Instron Universal Testing Machine (Model 4202, Canton, MA) equipped with a 50 N load cell was used to determine the viscosity index. Force-deformation curves of protein gels were recorded as a 8.5 mm diameter cylindrical plunger penetrated the gel at a constant rate of 50 mm/min to a distance 25 mm below the gel surface. The viscosity index or work to penetrate the gel was obtained by calculating the area under the curve as described by Smith *et al.* (1988).

The low speed centrifugation method of Nuckles *et al.* (1991) was used to the determine gel expressible moisture. A weighed 2 to 3 g sample of gel was placed on the filter paper, folded into a 50 ml centrifuge tube and centrifuged at 755 x g for 5 minutes at 2 °C. Expressible moisture was calculated by dividing weight gain of the filter paper by the original sample weight and multiplying by 100.

Scanning electron microscopy

Gels were prepared for SEM as described by Klomparens *et al.* (1986a). Protein gels were fixed with 2% (v/v) glutaraldehyde in 0.1 M Na phosphate buffer, pH 7.0, at 20 °C for 1 hour, followed by rinsing in 0.1 M Na phosphate buffer, pH 7.0, for 10 minutes. Dehydration of fixed specimens was performed in a graded ethanol series of 25%, 50%, 75%, 95% and 100% (v/v) for 10 minutes per step and stored in 100% ethanol overnight before use. Specimens were critical point dried using carbon dioxide in a critical point dryer (Model 010, Balzers, Liechtenstein), then mounted on 10 mm (diameter) x 5 mm (height) aluminum stubs (Electron Microscopy Sci., Ft. Washington, PA), and coated with

a thin layer of gold in an Emscope sputter coater (Model SC500, Ashford, England). All coated specimens were observed with a JEOL scanning electron microscope (Model JSM-35CF, Tokyo, Japan) at an accelerating voltage of 10 kV and 600 μm condenser lens.

Transmission electron microscopy

Specimens for thin-sectioning were prepared as described by Klomparens *et al.* (1986b). Salt soluble protein gels were fixed with 2% (v/v) glutaraldehyde in 0.1 M Na phosphate buffer, pH 7.2, for 2 hours at 20 °C and washed in 0.1 M Na phosphate buffer, pH 7.2, 3 times for 20 minutes each. Specimens were post-fixed with 1% (w/v) OsO_4 in the same buffer for 1 hour, stained with uranyl acetate (1 part H_2O:1 part saturated solution) for 1 hour, followed by rinsing in 3 changes of water for 20 minutes each. Dehydration was performed in a graded series of 25%, 50%, 75%, and 100% (v/v) acetone for 30 minutes per step, then soaked overnight in 100% acetone. The specimens were infiltrated successively in 25 and 50% epoxy resin in acetone, followed by 100% epoxy resin, embedded in 100% resin and held overnight. Thin-sectioning was carried out using an ultramicrotome (Model MT2, Sorvall, Norwalk, CT). Sections showing gold or silver interference colors were picked up on copper grids (Gilder, 300 mesh, Ted Pella, Inc., Redding, CA) and post-stained with Reynolds lead-citrate solution (Reynolds, 1963) for 1-3 minutes. All sections were viewed in a Philips transmission electron microscope (Model 201, Eindhoven, Holland) at 60 kV.

Samples were prepared for ultra-rapid freezing by placing a thin piece of gel and a spacer into the specimen carrier. The sample was frozen using a Gilky-Steahelin propane jet freezer (Model MF7200, R.M.C. Inc., Tucson, AZ) with a cryogen temperature of -190 °C, followed by etching and freeze-fracturing under vacuum in a modified Balzers apparatus (Model BA510). The sample was unidirectional-replicated with platinum and carbon deposited at an angle of 45°. The resultant replicas were cleaned with chromic acid, then transferred to carbon-coated grids. All specimens were observed in a Phillips transmission electron microscope (Model 201) operated at 80 kV and photographed at ± 6° tilt for stereo imaging. For micrograph interpretation, "strands" were defined as filamentous structures of 100 nm diameter or greater. "Filaments" had a diameter less than 100 nm.

Statistical design and analysis

A randomized complete block design was used to reduce variations due to the source of chicken. Main effects (treatment, replication) and interaction between treatments were calculated. The effects of pH were studied within each of three protein extractions (as a block). Blocks were not different ($P > 0.05$) from each other, thus one replicate was evaluated per block. A software program (MSTAT, version 5.0, Michigan State University, East Lansing, MI) was used for statistical analysis.

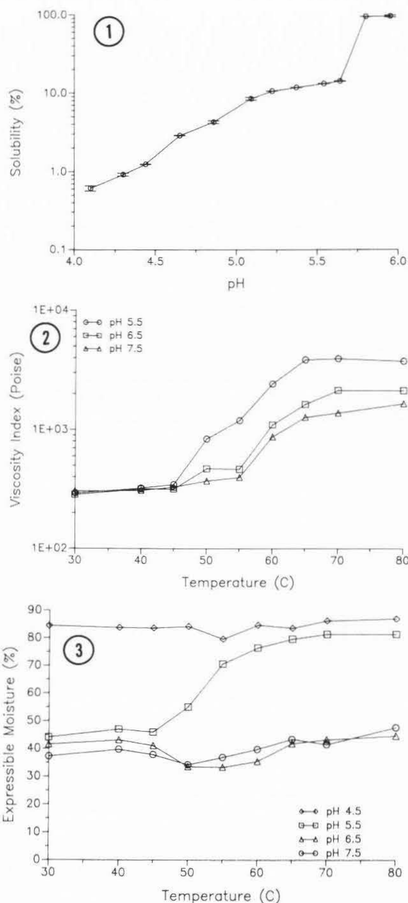
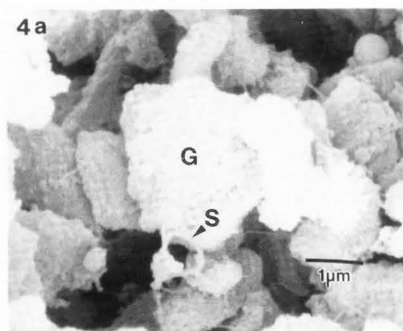
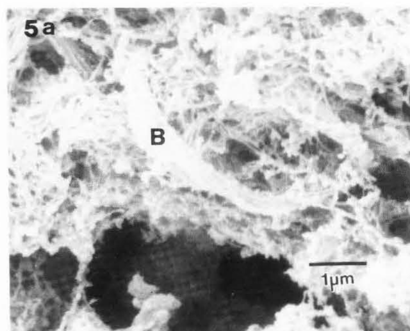


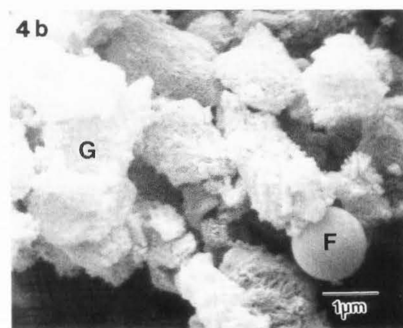
Figure 1. Effect of pH on the solubility of chicken breast salt soluble proteins (30 mg/ml) in 0.6 M NaCl, 50 mM Na phosphate.

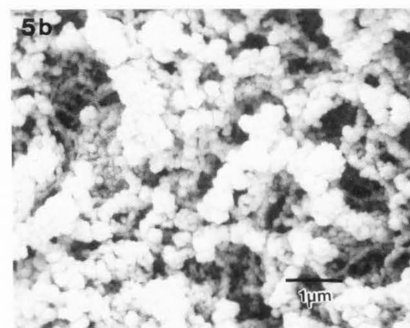
Figure 2. Effect of temperature on the viscosity index of chicken salt soluble protein (30 mg/ml) in 0.6 M NaCl at pH 5.5, 6.5 and 7.5. Standard error of the mean = ± 203.4, n = 6.

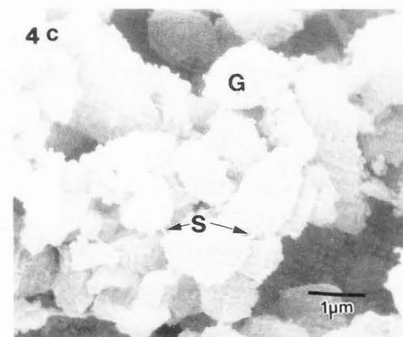
Figure 3. Effect of temperature on the expressible moisture of chicken salt soluble protein (30 mg/ml) in 0.6 M NaCl at pH 4.5, 5.5, 6.5 and 7.5. Standard error of the mean = ± 1.9, n = 6.

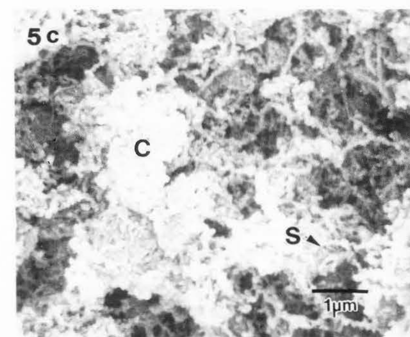




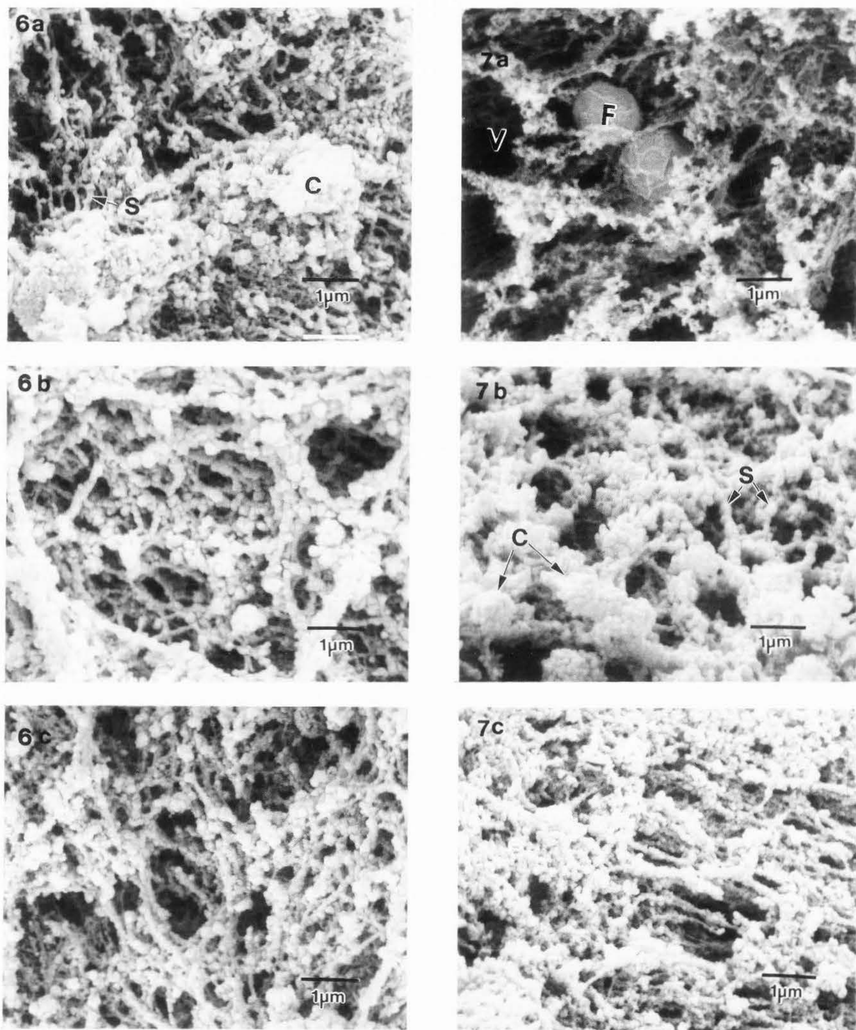








Figures 4-5. Scanning electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 4.5 (**Figure 4**) and pH 5.5 (**Figure 5**) heated to: a) 55 °C; b) 65 °C; c) 80 °C. S - protein strands; G - protein globules; F - fat globule; B - protein bundles; C - protein clusters.



Figures 6-7. Scanning electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 6.5 (**Figure 6**) and pH 7.5 (**Figure 7**) heated to a) 55 °C; b) 65 °C; c) 80 °C. C - protein clusters; S - protein strands; F - fat globules; V - voids.

Results

Functional properties

The SSP in 0.6 M NaCl, 50 mM Na phosphate buffer was 98% soluble at pH 5.8 and above (Fig. 1). Protein solubility decreased rapidly below pH 5.6 and was less than 1% at pH 4.3 and below. Solubilities in 0.6 M NaCl were 98%, 13%, and 2% at pH 6.5, 5.5 and 4.5, respectively.

Salt soluble proteins aggregated at pH 4.5 and formed a discontinuous gel which could not be measured by back extrusion. Protein gels prepared at pH 5.5 exhibited the highest viscosity index ($P > 0.05$) from 50 °C to 80 °C, followed by SSP at pH 6.5 and 7.5 (Fig. 2). The viscosity index of SSP at pH 5.5 increased from 45 °C and reached a plateau at 65 °C of 4000 poise. Gels prepared at pH 4.5 had expressible moisture above 80% at all heating temperatures (Fig. 3). Gels prepared at pH 6.5 and 7.5 had similar expressible moisture ($P > 0.05$), which changed less than 10% when heated from 30 °C to 80 °C. Expressible moisture of SSP gels at pH 5.5 increased ($P < 0.05$) from 46% at 45 °C to 80% at 70 °C. Changes in expressible moisture of SSP gels occurred at the same temperatures as the increase in viscosity index at pH 5.5.

Scanning electron microscopy

Salt soluble protein gels at pH 4.5 had an aggregated globular microstructure which did not change with heating temperature (Figs. 4a, b and c). The globules ranged from 1 to 3 μm diameter and some protein strands of 100 nm diameter were observed between the globules.

Heating temperature had a large influence on salt soluble protein gel microstructure at pH 5.5 and above. Gels prepared at pH 5.5 and heated to 55 °C formed filaments (ca. 30-70 nm in width) which were either associated side-by-side as bundles or were randomly oriented to form an irregular, filamentous network with large open spaces (Fig. 5a). The SSP gel structure at 65 °C contained small globular aggregates which were linearly oriented to form thick protein strands of approximately 400-800 nm diameter (Fig. 5b). Heating to 80 °C produced a network composed of aggregated globules of 1-2 μm diameter, connected by fine strands of approximately 100 nm diameter (Fig. 5c).

Aggregates composed of globules and connected by strands of 100-150 nm diameter were observed in SSP gels at pH 6.5 heated to 55 °C (Fig. 6a). An ordered, open continuous matrix of linear globular strands ranging from 200-400 nm were observed in SSP gels heated to 65 °C (Fig. 6b). The strands were thicker than those at 55 °C. The SSP strands decreased to 100-150 nm at 80 °C; however, the structure remained ordered with small voids (Fig. 6c).

An irregular, loose, lacy SSP network with large voids was observed at pH 7.5 and 55 °C (Fig. 7a). The narrow SSP strands appeared to be loosely coiled and were similar in diameter to strands observed at pH 5.5. Thick SSP globules ranging from 0.6 to 1 μm diameter were cross-linked by linear globular strands when gels were heated to 65 °C (Fig. 7b). The SSP strand diameters ranged from 100-200 nm and were larger than those at 55 °C, but narrower than strands at pH 5.5 and 6.5 at the same temperature. Shrinkage of SSP protein strand diameter was observed at 80 °C, but the network structure and SSP strand size were similar to that at pH 6.5 (Fig. 7c).

Transmission electron microscopy

Thin-sectioning. Thin-sectioning provided information at higher magnification on the organization of SSP filaments within the globular aggregates and strands observed by SEM. At pH 4.5, the SSP matrix contained large voids and discontinuous globular aggregates (1-3 μm in diameter) which were composed of loosely packed, long protein filaments of less than 20 nm diameter (Figs. 8a and 8b). Some of the filaments were associated in parallel bundles with cross-linking between bundles. Black spots on the micrographs were identified as lead which had precipitated during staining. As observed in SEM, further heating had little effect on overall appearance of SSP structure; however, SSP filaments became thicker and more compact at 80 °C.

The microstructure of SSP at pH 5.5 heated to 55 °C contained filaments and an indistinct globular matrix (Fig. 9a). This structure changed to a disordered, more compact, locally dense structure when heated to 80 °C (Fig. 9b) which corresponded to the aggregated clusters viewed by SEM (Fig. 5c). Individual structures within the large clusters were difficult to identify, but some parallel filaments were observed with a diameter less than 30 nm.

A continuous lacy network with small voids was observed in SSP gels at pH 6.5 when heated to 55 °C (Fig. 10a). The continuous network contained strands of 30-150 nm diameter which appeared to be composed of cross-linked filaments. The strands were connected by fine filaments of less than 10 nm diameter (Fig. 10a). When heated to 80 °C, a more ordered continuous network composed of denser SSP strands of 120 nm diameter, again cross-linked by fine filaments of less than 10 nm diameter was observed (Fig. 10b). Microstructures of thin-sectioned SSP gels heated at pH 7.5 (Figs. 11a and 11b) were similar to those observed at pH 6.5. At 55 °C, SSP strands were thicker (130-200 nm diameter) and more closely packed compared to gels at pH 6.5.

Ultra-rapid freezing. Microstructures of SSP gels heated to 80 °C were examined by TEM using the ultrarapid freezing method. The three dimensional image of SSP at pH 4.5 exhibited two types of structure: globules (1 μm diameter; Fig. 12a) and small aggregates connected by long coiled filaments (Fig. 12b). The coiled SSP filaments appeared to be composed of two intertwined filaments of 50 nm diameter and were loose in structure. Globular aggregates connected by SSP strands were observed by SEM.

Gels at pH 5.5 contained a regular lattice network

of 30 nm filaments and small voids interrupted by irregular, dense regions of globules (Fig. 13). The dense regions may correspond to aggregates observed in SEM and thin-sectioned TEM samples (Figs. 5c and 9c). Gels prepared at pH 6.5 and 7.5 contained highly ordered, continuous lattice networks, composed of 30 nm coiled SSP filaments with some dense regions of more highly aggregated protein (Figs. 13b and 13c). Gel microstructures observed at pH 6.5 and 7.5 using ultra-rapid freezing techniques contained regular, highly cross-linked stranded networks which were also observed by thin-section TEM and SEM techniques.

Discussion

Gel microstructure depends on the charges of native proteins in solution during heating. Low repulsive forces between molecules lead to aggregation whereas high repulsive forces suppress random aggregation to form an ordered structure (Barbu and Joly, 1953; Hermansson, 1986). Salt soluble protein at pH 4.5 was insoluble (less than 2% solubility in 0.6 M NaCl) and formed a highly aggregated, globular gel structure with few large voids and poor water-holding abilities, suggesting this pH is near the isoelectric point of chicken breast actomyosin. Temperature had little effect on gel properties or structure at pH 4.5, suggesting that heating did not change the highly aggregated protein networks formed prior to heating. Results agree with Wang *et al.* (1990) who reported no change in storage or loss moduli with temperature for SSP gels at pH 4.5 heated from 30 to 80 °C.

The SSP at pH 5.5 was partially soluble in 0.6 M NaCl and changed structure to form a gel with heat. Viscosity index began to increase and water holding ability to decrease at 45 °C and above indicating protein denaturation and aggregation to form a cross-linked matrix. The high expressible moisture observed in gels at pH 5.5 was indicated by large voids in the protein matrix observed by SEM and TEM with ultra-rapid freezing. Gels at pH 5.5 had the highest viscosity index at all temperatures above 45 °C. Gels may have had the highest viscosity index for several reasons: a) the insoluble proteins may act as fillers to reinforce the gel structure (Tolstoguzov, 1986); b) stronger local interactions due to relatively lower repulsive forces among protein molecules may increase strand size; c) effective increase in protein concentration due to syneresis of fluid from the gel; or d) more developed gel network at lower temperatures due to earlier structural transitions. According to SEM micrographs at 65 °C and 80 °C, gels at pH 5.5 had thicker SSP strands and larger aggregates than those at pH 6.5 and 7.5. The dense regions of gel structure observed in ultra-rapid-freezing images may also explain the high viscosity index observed. The pH for maximum gel rigidity at 0.6 M KCl has been reported at pH 5.4 to 5.6 for chicken breast myosin (Asghar *et al.*, 1984; Morita *et al.*, 1987) which corresponds to our results with SSP.

Salt soluble protein gels at pH 6.5 and 7.5 exhibited similar changes in water-holding capacity and viscosity index with temperature. Viscosity index of SSP gels at pH 6.5 and 7.5 increased from 55 °C which was 10 °C higher than that observed for gels at pH 5.5. Wang *et al.* (1990) reported that the temperatures at which initial changes in storage and loss moduli of SSP occurred during heating were almost identical at pH 6.5 and 7.5, but were higher than those at pH 5.5, suggesting SSP aggregated at a faster rate at the lower pH. The lowest expressible moistures were observed in SSP gels at pH 6.5 and 7.5 which also had the most uniform and continuous gel matrices as observed by SEM and TEM techniques, suggesting this type of matrix is important to gel water holding ability.

Temperature had a large influence on viscosity index, expressible moisture and gel microstructure. When heated to 80 °C, SSP strands at pH 5.5, 6.5 and 7.5 had decreased diameters compared to 65 °C due to heat-induced shrinkage. Several authors have reported decreases in muscle protein gel strength above a certain maximum temperature (Montejano *et al.*, 1984; Wu *et al.*, 1985).

Myosin molecules are present as monomers when ionic strength is above 0.3 μ at neutral pH and form filaments when ionic strength or pH is decreased. Kaminer and Bell (1966) observed short myosin filaments and monomers at 0.3 M KCl, pH 6.5, but myosin monomers predominated at pH 8.0. Yamamoto *et al.* (1988) proposed that length of myosin filaments before heating determined myosin gel structure and strength. In our study, long filaments were observed at pH 4.5 in the thin-sectioned images at 55 and 80 °C. These long filaments were not observed at higher pH's, suggesting that the polymerization status of SSP before heating might also influence gel structure.

Conclusion

The pH influenced SSP solubility in 0.6 M NaCl, 50 mM Na phosphate buffer and the gelation process which resulted in different water-holding capacities, viscosity index and microstructure. The examination of SSP gels using different electron microscopic techniques aided the understanding of matrix formation at different pH's and heating temperatures. More detailed studies of the role of proteins in structural development during heating will help to optimize meat processing conditions and quality.

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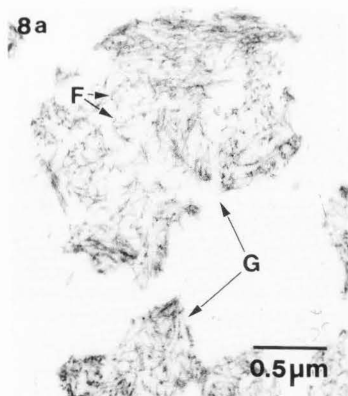

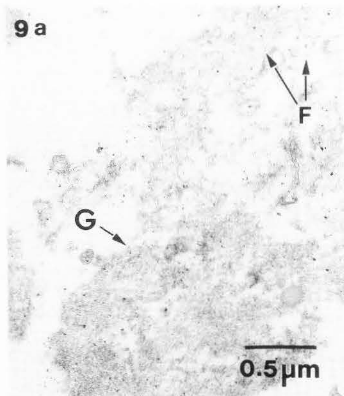

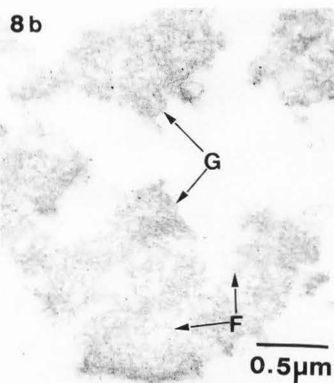

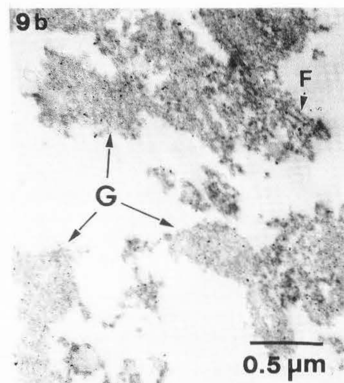

Figure 8. Thin-sectioned transmission electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 4.5 heated to a) 55 °C; b) 80 °C. G - protein globules; F - protein filaments.

Figure 9. Thin-sectioned transmission electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 5.5 heated to a) 55 °C; b) 80 °C. G - protein globules; F - protein filaments.

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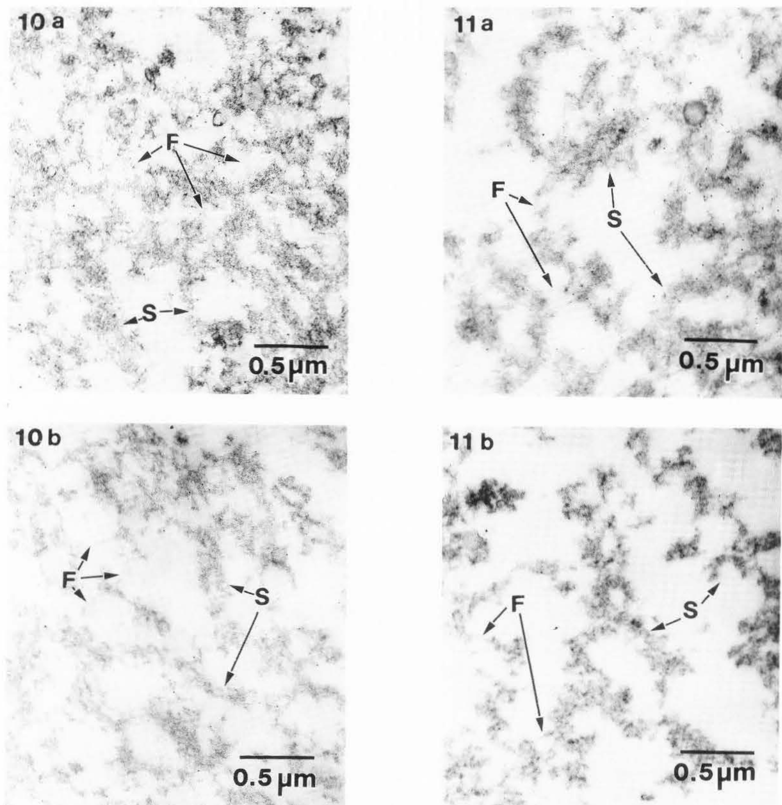

Figure 10. Thin-sectioned transmission electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 6.5 heated to a) 55 °C; b) 80 °C. F - protein filaments; S - protein strands.

Figure 11. Thin-sectioned transmission electron micrographs of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 7.5 heated to a) 55 °C; b) 80 °C. F - protein filaments; S - protein strands.

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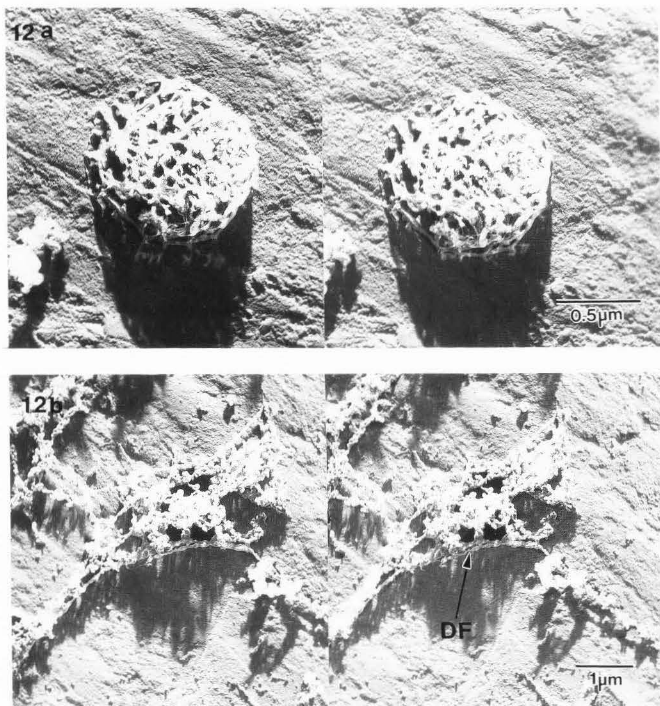

Figure 12. Stereo images of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl at pH 4.5 heated to 80 °C prepared for transmission electron microscopy by ultrarapid freezing. **a**. Globules. **b**. Small aggregates and filaments. DF - coiled double filament.

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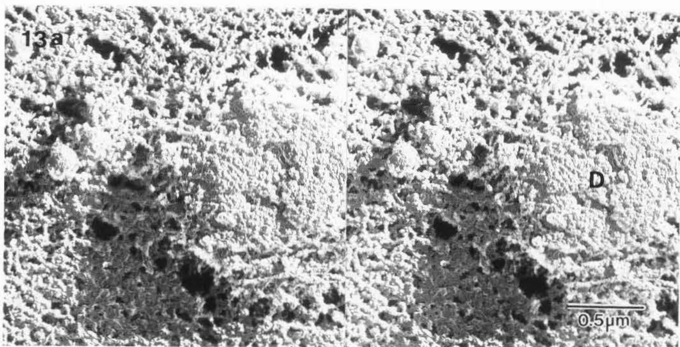
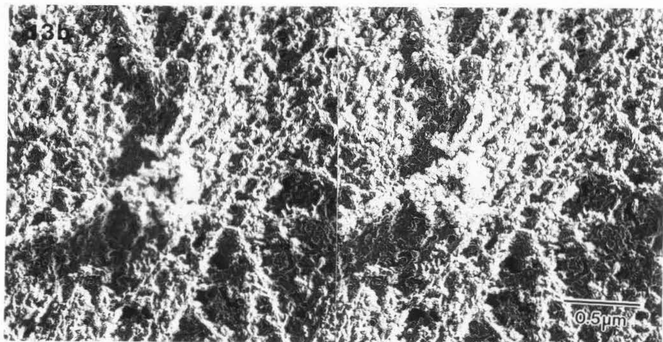
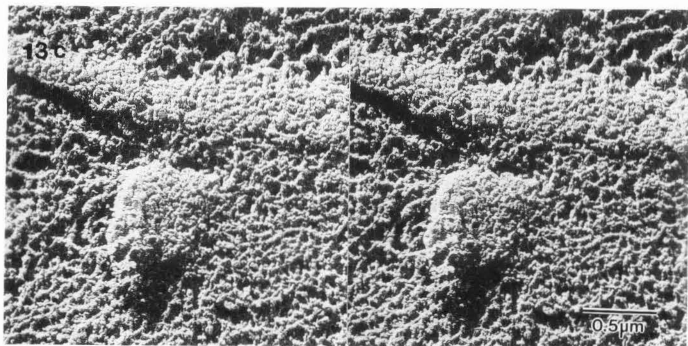


Figure 13. Stereo images of chicken salt soluble protein gels (30 mg/ml) in 0.6 M NaCl heated to 80 °C prepared for transmission electron microscopy by ultra-rapid freezing. **a**. pH 5.5. **b**. pH 6.5. **c**. pH 7.5. D - dense globular region.





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Discussion with Reviewers

C.M. Lee: Can you comment on which preparation condition will yield the most desirable gel in terms of solubility and texture based on the findings?

Authors: The term "desirable gel quality" needs to be defined clearly because it depends on the requirements of different types of meat products. Based on our studies, gels at pH 5.5 had the highest viscosity index, whereas gels at pH 6.5 and 7.5 exhibited better water holding ability.

C.M. Lee: Can you rationalize the selection of different pH ranges and intervals?

Authors: The pH's of most meat products range from pH 5.5 to 6.5. Extreme conditions are of interest since proteins may respond differently and help us to understand protein behavior.

C.M. Lee: Is viscosity index the right choice for measurement of functional or viscous properties? Should viscosity index be inversely related to expressible moisture; i.e., the more functional the protein sol, the lower the expressible moisture?

Authors: According to our previous study of SSP gel properties measured by dynamic testing (Wang *et al.*, 1990), both storage (elastic properties) and loss (viscous properties) moduli increased and paralleled each other during heating. Back extrusion is an empirical rheological test. Viscosity index is determined from the force required to penetrate the gel and can be used to compare weak gels that cannot maintain a free standing rigid structure, such as a cylinder or dumbbell. There is no reason for viscosity index and expressible moisture to be inversely related under all conditions.

C.M. Lee: Is there any special preference towards or advantages of Klomparens's procedure over others on SEM and TEM?

Authors: The procedures described in the laboratory manual by Klomparens *et al.* (1986) are traditional methods for specimen preparation for SEM and TEM. These methods are often used in our laboratory and give us satisfactory results.

E.A. Foegeding: Can the authors give the relative percentage of myosin and actin in the SSP fraction?

Authors: This is an excellent point. We used a densitometer to estimate the relative quantities of myosin and actin on sodium dodecyl sulfate polyacrylamide gels stained with Coomassie blue dye. Muscle proteins do not absorb this dye equally, and thus the ratio is an estimate. Quantification of other proteins in the electrophoresis gel would lead to larger errors in the estimate.

E.A. Foegeding: Are the samples gelled at 30 °C?

Authors: The samples flowed on standing at this temperature.

E.A. Foegeding: Yamamoto *et al.* (1988) showed that long, thick myosin filaments formed gels with greater rigidity than smaller filaments. In this study, the opposite results were shown. Why?

Authors: We cannot compare our results directly to Yamamoto *et al.* (1988) as we did not observe the filamentous structure of our SSP solutions prior to heating.

We are just suggesting that the gel structure after heating may be influenced by the filamentous nature of the proteins prior to heating. It has been reported that pH influences the filament forming abilities of myosin. More research is needed in this area.

K. Samejima: There seems to be no differences in the results of viscosity index (Fig. 2) and expressible moisture (Fig. 3) between 65 °C and 80 °C. However, there is a drastic distinction in their morphological features (Figs. 5b and 5c). What is your opinion about this point?

Authors: We do not know why there were no differences in viscosity index or expressible moisture as the microstructure changed from 65 °C to 80 °C. Perhaps the methods used were not sensitive enough to pick up differences caused by the slight changes in microstructure.