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Gelation properties of porcine muscle proteins

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Gelation properties of porcine muscle proteins

Camou Arriola, Juan Pedro, Ph.D.

Iowa State University, 1989

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Gelation properties of porcine muscle proteins

by

Juan Pedro Camou Arriola

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

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Departments: Food Technology
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For the Graduate College

Iowa State University
Ames, Iowa

1989

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DEDICATION

To my wife, María del Carmen, for her love, support, patience, for taking care of our children, and for sharing with me the good and the hard times of a graduate student. I would not be able to accomplish this dream without her at my side. I love you. To my daughter, Mary Carmen, for your love, for those long sleepless nights and for being the first one in calling me daddy. To my son, Juan Pedro, for bringing more light to my life. To my parents, Edgardo and Eleonor, for their love and for teaching me the good things of this life. To my brothers and sisters, my parents in-law and their families for sharing with me and María del Carmen the pain of being away from home all these years.

To God, my creator, for all these riches that I have had in my life and I do not deserve. Thank you.

INTRODUCTION

Myosin, actomyosin, and their combination with other myofibrillar protein fractions have been commonly used as model systems to study the rheological properties of extracted muscle protein gels. Myosin and actomyosin are generally considered to contribute most of the quality and strength of binding in sausages and in model systems. Texture and appearance of these products or of model systems are altered by changing the environmental conditions that surround these myofibrillar proteins. Environmental changes often will result in a dramatic decrease in protein functionality. This decrease affects gelation, water binding, fat binding, and the general stability of the meat product or model system. Factors such as the physicochemical state of myofibrillar proteins, pH, salt, and thermal treatment might exert a positive or negative effect on protein functionality.

The physicochemical state of myofibrillar proteins is very important in meat processing since it directly affects protein solubility and protein functionality. With more soluble protein in the system more protein is available for the different types of protein interactions needed to stabilize the system. These protein interactions include: protein-protein, protein-water, and protein-lipid. Pale, soft, exudative pork (PSE) is a condition that results in poor protein functionality and protein solubility as a result of early muscle postmortem chemical changes. These changes, a combination of high temperature and high content of lactic acid, induce protein denaturation.

The direct effect of pH and salt (NaCl) concentration on protein solubility is well known. Protein solubility increases at higher pH (6.0-7.0) while it decreases

at lower pH (5.4-6.0). Maximum protein solubility occurs when salt concentration is in the range of 6-8%; however, this has a detrimental effect on flavor. Two percent to 3% salt in the system is the most widely used concentration by the meat industry. Less than 1.5-2% dramatically decreases protein solubility, thereby causing a bland, mushy product.

Gelation of myofibrillar proteins in meat products or model systems results from protein-protein interaction induced by application of heat. Caution has to be taken when selecting or applying temperature to the system. Coagulation or gelation of myofibrillar proteins result from protein-protein interactions during heat treatment. Coagulation is the random aggregation of heat denatured protein molecules favored by fast heating rates. Gelation is an ordered aggregation that occurs during protein denaturation with formation of a continuous three dimensional protein network; it is favored by conditions such as a slow heating rate.

Non-meat proteins have been used in meat products to improve yield and to decrease raw material cost. However, addition of these non-meat proteins will have an effect on traditional product eating quality characteristics, such as texture and flavor. Caution should be used when these non-meat proteins are added to the system. Non-meat proteins have lower functionality when compared to myofibrillar proteins. Their functionality, however, can still aid texture, water binding, fat binding and overall stability of weak formulations if they are used according to manufacturers' instructions to maximize their functional properties.

Many of the rheological studies on model systems have been conducted with myosin or actomyosin systems at low-protein-concentration (below 10 mg/ml) using constant heating temperatures. Salt concentration and pH have

been varied from below to above the average conditions practiced in meat processing operations. Little information is available on textural properties of extracted salt-soluble muscle protein gels at high protein concentration (>10 mg/ml) and containing both sarcoplasmic and myofibrillar protein fractions. The use of progressively increasing heating rates instead of constant heating rates for gel formation also has been little investigated.

The objective of this study was to investigate the effect of heating rates, protein concentration, PSE condition, and addition of non-meat proteins on quality characteristics of thermally formed protein gels that closely resemble processed meat products. The extracted salt-soluble protein mixture in this study contained both sarcoplasmic and myofibrillar protein fractions that closely resemble the extracted fraction that occurs in processed meat products. Water retention of gels was evaluated to assess potential yield effects. Finally, gel electrophoresis was utilized to confirm initial protein composition of salt-soluble protein extracts and to study the protein profile of soluble protein in the exuded water after compression of heat-set gels.

LITERATURE REVIEW

Skeletal muscle or meat can be converted to a wide variety of meat products. These can be classified depending to their degree of comminution as either noncomminuted and comminuted meat products. Noncomminuted meat products gain their distinguishing characteristic by being prepared from whole intact muscle like hams and bacons. On the other hand, comminuted meat products involve reducing of the raw meat materials into smaller meat pieces. Depending on the degree of comminution the result will be coarse ground meat products like summer sausage or salami, and fine or "emulsified" meat products like frankfurters or bologna.

There is a typical sequence of steps involved in the processing of comminuted meat products. This sequence involves reduction of the raw meat material to the desired particle size through grinding. Addition of salt (NaCl) along with mechanical agitation like mixing or chopping (in a silent cutter) will result in partial solubilization of myofibrillar proteins. This will yield a thick paste (like frankfurter batter) or thick blend (when making coarse products) or thick exudate (hams after tumbling). What they all have in common at this step is that they are tacky due to protein solubilization. A heating process follows and this establishes a gelled network of proteins suitable to stabilize chemically and physically both water and fat, and at the same time add the characteristic product texture.

It can be deduced that there are two principal steps involved in the making of meat products: protein extraction and protein gelation. These two steps, if performed under adequate conditions, will result in a stable meat product. Any

condition that detrimentally affects these two steps will result in probable product failure (Acton and Dick, 1984).

Muscle Proteins

Skeletal muscle proteins are classified into myofibrillar, sarcoplasmic and stromal proteins. Myofibrillar proteins comprise about 50 to 55% of the total protein content and are composed mainly of myosin, C-protein, M-protein, actin, tropomyosin, troponin complex and α-actinin among others. Sarcoplasmic proteins comprise about 30 to 34% of the total protein content and are composed mainly of myoglobin and the glycolytic enzymes. Stromal proteins comprise about 10 to 15% of the total protein content and they are referred to as the connective tissue proteins (Morrissey et al., 1987).

Myofibrillar and stromal proteins form part of a very complex structure in skeletal muscle. Stromal or connective tissue proteins provide structural support to maintain muscle integrity and transmit contraction of the muscle fibers to the skeleton. The arrangement of connective tissue can be seen when muscle is cross sectioned. An entire muscle is usually surrounded by a heavy connective tissue sheath called the epimysium. Muscle is divided into smaller groups of cell fibers called bundles surrounded by a thinner connective tissue called perimysium which appears to be continuous with the epimysium sheath. Each cell fiber is surrounded by very thin connective tissue layers called the endomysium which also appears to be connected to the perimysium.

Skeletal striated muscle fibers are elongated, multinucleated, and more or less tubular cells. They are encased by a delicate membrane immediately located under the endomysium. These muscle fibers are composed of myofibrils

which contain the myofibrillar protein. The myofibrils are embedded in the cytoplasm of the fiber, which is called the sarcoplasm. Sarcoplasm contains the sarcoplasmic protein, water and other components (Cassens, 1987).

Protein Extraction

Grinding and chopping disrupts or partially destroys muscle and cellular structure. Sarcoplasmic proteins are released from inside of the muscle cell to the exterior or surface of the meat particle. Once reduction has been accomplished salt (NaCl) will aid in extracting and solubilizing myofibrillar proteins. Also, phosphate may be used to accomplish the removal of more myosin molecules from their ultrastructure by dissociating the actomyosin complex. There will be a combination of myosin molecules, actomyosin, other myofibrillar proteins and sarcoplasmic proteins on the surface of the meat particles. Connective tissue proteins are insoluble under these conditions and are of limited functional value. Soluble proteins act as emulsifying agents by coating surfaces of the dispersed fat particles. This soluble protein fraction will form a stable heat-set gel that will very efficiently bind fat and water in a processed meat product. The heat-set three dimensional protein network entraps and binds both water and fat, thus achieving permanent stability for the meat product. The protein network also may stabilize the finely or coarsely reduced meat particles by interacting with myosin filaments located within fragments of muscle cells on or near the surface of the meat particle (Tarrant, 1982). To form a successful meat product, the system has to have the conditions that allow a balance of protein interactions. Too little myosin extraction will create a product with high water loss and mushy or grainy texture due to the lack of protein-protein

interactions. Too much myosin will allow protein aggregation and batter failure. These interactions must contain the fat and water yet retain the elastic texture needed to maintain the product from failure during cooking, storage, freezing, and recooking in the home (Whiting, 1988).

Protein Functionality

Skeletal muscle proteins are characterized by the basic functional properties of gelation, water binding, and fat binding. This is the result of muscle protein participating in three classes of interactions: protein-water interaction, protein-lipid association and protein-protein aggregation. They are interrelated and depend on the antemortem and post mortem biochemical state of the muscle and on environmental conditions surrounding the muscle proteins. Conditions like salt concentration, pH, and the temperature profile (which usually shifts upward from approximately 0°C as initial temperature to about 71°C final cooked temperature) are some of the most critical factors.

Myosin is the most important component of the soluble protein fraction with respect to these basic functional properties (Fukazawa et al., 1961a, 1961b, 1961c).

Protein Gelation

Gelation is the process where denatured molecules aggregate to form an ordered protein network. This protein gel formation occurs by means of protein denaturation under strictly defined conditions. Gel strength or rigidity is generally used as a criterion for evaluating gel characteristics, and it is one of the most important parameters for studying protein functionality in gelation. Gel strength,

as well as water and fat binding are direct reflections of the nature of the extracted protein fraction.

Myosin

Myosin is composed of tail and head portions and they play different roles in the heat-induced gelation of muscle minces and of model systems (Ishioroshi et al., 1981; Samejima et al., 1981; Samejima et al., 1983; Samejima et al., 1984). In fact myosin gelation involves two stages (Samejima et al., 1981; Acton and Dick, 1984). In the first stage the head portion of the myosin molecule aggregates with neighboring myosin heads, while the second step involves interaction of myosin tail portions. Samejima et al. (1981) used chymotrypsin to cleave myosin molecules (from longissimus thoracis of rabbit) into S-1 and rod subfragments. They compared the heat-induced gelation of these two subfragments to intact myosin molecule in 0.6M KCl, pH 6.0 and at a protein concentration of 4.5 mg/ml. Gels made of myosin and myosin rods were firm enough to remain in the cell upon its inversion. Gels made of S-1 subfragments showed very poor gelation and extremely poor water retention upon heating. The same authors also found that gels of myosin and the rod were made of an extended protein network, whereas bead-like protein aggregates were observed in gels made of S-1 subfragments. When mixtures of S-1 and rod fragments were gelled they failed to form the same type of gels as formed by the intact myosin protein. This mixture revealed morphological characteristics similar to those of the rod alone. Samejima et al. (1981) found that gels made of intact myosin molecules had the maximum rigidity value (1,750 dyne/cm^2), S-1 gels had the lowest (300 dyne/cm^2), rod gels exhibited an intermediate value (1,050

dyne/cm^2), and gels of a mixture of S-1 and rod (525 dyne/cm^2) together failed to restore the rigidity of the gel made of myosin alone. These gel-strength measurements were conducted at same protein level of 4.5 mg/ml when they were heated at 70°C for 20 minutes. It has been found that myosin and actomyosin during gelling show two transition temperatures (Samejima et al. 1981; Acton and Dick, 1984). In other words, gelation occurs in two stages. The first transition temperature or stage involves aggregation of the globular head region of the myosin molecule. This aggregation occurs early at the heating temperature range between 30°C and 50°C (Kawakami et al., 1971; Ishioroshi et al., 1979; Samejima et al., 1981; Ziegler and Acton, 1984). These interactions apparently are associated with oxidation of -SH groups that reside in that region, however, noncovalent bondings also could be involved (Acton and Dick, 1984). The second transition temperature occurs at a temperature higher than 50°C (Samejima et al., 1972, 1976; Ishioroshi et al., 1979; Acton and Dick, 1984). This is associated with the unfolding of the helical tail portion of myosin. These tail-to-tall interactions are apparently stabilized by hydrophobic interactions, since hydrophobic residues are exposed to the surface of the myosin rod when the rod starts unfolding due to the effect of temperature. Both interactions contribute to the formation of the three-dimensional protein network formation, however, myosin rod interactions are essential in the formation of the protein network during gelation (Ishioroshi et al., 1981), since it is not until myosin rod undergoes helix-coil transformation that cross-linking of these segments starts having a direct effect on gel strength (Acton and Dick, 1984). The aggregation of myosin heads is important, however. They associate to form aggregates that act as "super-junctions" where the filament network interlinks (Samejima et al., 1981)

confering higher stability and gel strength to the system. In terms of gel strength, Ishioroshi et al. (1979) and Yasui et al. (1979, 1980) reported that myosin and actomyosin samples started to develop gel rigidity at about 40°C and reached a maximum at about 60°C.

These transition temperatures were corroborated by studying the S-1 and rod subfragments from myosin (Samejima et al., 1981). Both S-1 and the rod showed single transition temperatures at 43°C and 55°C, respectively. S-1 subfragment interactions involved oxidation of -SH groups like those from intact myosin molecule during the first transition temperature. Heat-induced gelation of the rod fraction was independent of oxidation of -SH groups, however, rod-to-rod associations were stabilized by hydrophobic interactions. Aromatic amino acid residues were transferred from the moderately hydrophobic environment inside the myosin molecule to a more polar environment on the surface of the molecule inducing an irreversible change on protein conformation.

Ishioroshi et al. (1981) cleaved myosin molecules from rabbit longissimus thoracis muscle into heavy meromyosin (HMM) and light meromyosin (LMM). HMM is the low ionic strength soluble fragment that contains the double head fragment and a part of helical tail region. LMM is the low ionic strength insoluble fragment, 80 nm in length, that contains a part of the helical tail region. Under conditions of 0.6M KCl, pH 6.0 and 4.5 mg/ml proteins, LMM had similar rigidity values (slightly lower) than that of the rod (LMM + S-2 fraction, which length is 140 nm), 940 and 1050 dyne/cm^2, respectively. This shows that elimination of the S-2 fraction from the myosin total rod does not affect drastically the gelling ability of the tail region of the molecule. On the other hand, HMM had a rigidity value of about 790 dyne/cm^2 (at 60°C), which was higher than that of the S-1

fraction (450 dyne/cm^2). This shows that addition of the S-2 tail fraction to the S-1 (together make the HMM) globular head fractions improved the very poor gelling ability of the latter fraction.

LMM had only one transition temperature at 53°C (Ishioroshi et al., 1981), like myosin rod (Samejima et al., 1981). LMM molecule exists as monomers or dimers under high ionic strength and pH 6.0. Under these circumstances it plays a major role during heat gelation of myosin, since it is able to unfold and to associate to form an irreversible three-dimensional protein network.

Further studies on myosin gelation were carried on by Samejima et al. (1984) by studying the gelation behavior of myosin heavy chains (MHC) from rabbit leg muscles. Gel strength was of almost equal magnitude for both myosin molecules and MHC. These authors suggested that gel strength depended only on MHC, and that the presence or absence of light chains (LC) did not contribute to the system's rigidity. This agrees with Locker's proposition (Locker, 1956) that MHC coagulates while LC dissociates and solubilizes during the heating of intact myosin molecules. MHC formed a very weak gel when heated up to 35°C. However, when temperature was raised to 60°C gel strength increased almost exponentially. This behavior is similar to the intact myosin molecule. MHC gelation also showed two transition temperatures at 42°C and 55°C. The first transition temperature started at 35°C and was completed at 40°C. The second transition temperature occurred in the temperature range of 40°C to 60°C. These transition temperatures correspond to the ones observed for intact myosin molecules by Samejima et al. (1981) where the first transition peak was due to head-to-head interactions and the second due to tail-to-tail interactions.

All of these studies on the heat-induced gelation phenomenon of intact myosin and myosin subfragments (rod, LMM, HMM, S-1, and S-2) point out to the most important factor that integrity of the myosin molecule is essential for the optimum formation of the three-dimensional protein network.

Actomyosin

Samejima et al. (1969) studied the effect of F-actin in the gelation of myosin isolated from rabbit muscle at 0.6M KCl, pH 7.0, and heating the protein sample (which protein concentration ranged from 1 to 17 mg/ml) at 60°C for 30 min. They found that F-actin coagulated upon heating, but it failed to form a gel. However, F-actin improved gel strength when complexed with myosin.

Nakayama and Sato (1971a) studied the rheological properties of myosin A (myosin molecules), myosin B (actomyosin with native tropomyosin complex), and myosin with F-actin from white leghorn hen muscle in 0.6M KCl at pH 7.0 and heated at 80°C for 30 min. They found that rigidity of samples from myosin A (protein concentration 6.9%) was lower than that from myosin B (protein concentration 6.9%) as it was reported earlier by Samejima et al. (1969). Rigidity of heat-set gels decreased when ATP was added to myosin B samples. This decrease was linked to the presence of actin. They concluded that even though myosin A was the protein that contributes most of the binding properties, there was an interaction between myosin A and actin that was contributing to the rheological properties of the heat-set gel. In a later study Nakayama and Sato (1971b) indicated that the presence of F-actin in the appropriate ratio to myosin A was necessary to improve binding quality of the myosin B complex.

Yasui et al. (1980, 1982) studied the effects of F-actin at different ratios on myosin gelation in 0.6M KCl and pH 6.0 for a protein concentration of 5 mg/ml. They found that F-actin alone did not show any sign of gelation, although it precipitated with liberation of water when temperature was increased above 50°C. This is supported also by a later work by Sano et al. (1989) where they found that F-actin upon heating did not form an elastic gel but rather turned into a curdy matter. However, Yasui et al. (1980) found that F-actin had a marked influence on the heat induced gelation of myosin when F-actin was mixed with myosin. The molar ratio of myosin to F-actin (M:A) ranged from 0 to 2.7. Rigidity increased from about 250 dyne/cm^2 measured at 70°C for F-actin alone, to a maximum rigidity of about 5,000 dyne/cm^2 for a M:A of 2.7. Rigidity reached a minimum of 1,500 dyne/cm^2 for a M:A ratio of 0.26 at 70°C. When the M:A ratio was increased further, rigidity values decreased to the level of that of myosin alone, which was about 2,000 dyne/cm^2. In a later study Yasui et al. (1982) postulated that the enhancing effect of F-actin on gelation properties of myosin was carried by the limited amount of F-actomyosin formed in the system, which cross-links the bound myosin tail portions with their counter parts from free myosin molecules. They reported maximum gel rigidity for the myosin-actomyosin system occurred with 80% free myosin and 20% F-actomyosin (fully decorated) at pH 6.0, 0.6M KCl and heat-set at 65°C. However, a later study by Ishioroshi et al. (1983) showed that addition of F-actin (0.5 to 3.0 mg/ml) to the myosin system at low ionic strength (0.2M KCl) caused a decrease in the heat-induced gel strength of the myosin system. They suggested that the synergistic effect of actin on myosin gelation is either dependent on ionic strength or determined by the state of myosin molecules at a specific ionic strength.

When Yasui et al. (1980) added ATP to the myosin-F-actin mixture, rigidity decreased below that of the myosin control. This work suggested that myosin has to be bound to actin before the heat treatment in order to have the augmentation effect on rigidity of the heat set gels. In a different study, Ishioroshi et al. (1980) studied further the effect of myosin-actin mixtures on rigidity of heat-induced gels. They chemically modified myosin or actin by addition of ATP or PPi before thermal treatment. It was found that ATP and PPi dissociated the actomyosin complex into myosin and actin at high ionic strength (0.6M KCl and pH 6.0), and agreed with the findings of Yasui et al. (1980) where rigidity of actomyosin was remarkably decreased to values well bellow of those of control myosin without actin and polyphosphates. They (Ishioroshi et al., 1980) stated that this decrease of rigidity of heat-set gels of actomyosin in the presence of ATP and PPi (inorganic pyrophosphate) was caused by the modification of the electrostatic nature of myosin. The binding of these compounds to myosin caused an increase in the net charge and a change on myosin conformation exerting a negative influence on gel rigidity. However, rigidity was restored by hydrolysis of those diphosphates (ADP and PPi) either by inorganic pyrophosphatase (PPase) or by adenylate kinase.

Scanning electron microscopy (SEM) by Yasui et al. (1980, 1982) showed that gels made of myosin alone were structural networks with globular projections. These networks were transformed into more homogeneous and smoother thread-like three-dimensional networks with a decreased pore size upon raising the amount of actomyosin complex. As the M:A ratio was decreased by increasing the amount of F-actin, the structural network of the actomyosin complex became more like the actin precipitates. The morphology of the actin

precipitates were bead-like with large spaces between the aggregates which failed to efficiently trap water. Ishioroshi et al. (1980) conducted SEM studies and found that the well structured three-dimensional protein network of actomyosin disappeared when ATP or PPi were added, however, after hydrolysis of ATP and PPi the three-dimensional protein network reappeared.

Yasui et al. (1982) studied the effect of myosin subfragments (S-1, HMM, rod, and LMM) on heat-induced gelation in the presence of F-actin or F-actomyosin. They reported no effect on the gel strength of the heat-induced gel. Subfragments S-1 and HMM bound to F-actin. However, the rod and LMM subfragments failed to interact with F-actin since they are devoid of the binding site which resides only on the head part of myosin molecules. Although S-1 and HMM can interact with F-actin they lack the necessary tail portion for network formation. On the other hand, rod and LMM can interact with each other but they can not bind to F-actin, failing to form a three-dimensional network. This agrees with the findings of previous authors (Ishioroshi et al., 1981; Samejima et al., 1981; Samejima et al., 1984) where integrity of the myosin molecule is essential for the optimum formation of the three-dimensional protein network not only for a system with just myosin molecules but also when the system contains F-actomyosin.

Tropomyosin and Troponin

Nakayama and Sato (1971b) studied the effect of native tropomyosin (a complex of tropomyosin and troponins) in myosin B (actomyosin) from hen muscle. They found that native tropomyosin along with F-actin increased the binding quality and water holding capacity of myosin A.

Cheng et al. (1979) studied gel texture of heat-induced fish gels and found that degradation of tropomyosin during thermal processing had lower rigidity values. They proposed that the effect of thermal processing on gel textures are closely related to the extent of tropomyosin degradation.

Samejima et al. (1982) do not agree with these previous findings. They studied the effects of tropomyosin (TM) and troponin (TN) on heat-induced gelation of myosin. Natural (with TM and TN) and desensitized (without TM and TN) actomyosin systems from rabbit skeletal muscles at pH 6.0, 0.6M KCl and heated at 65°C for 20 minutes were studied. No significant differences in gel strength were reported between these two systems. SDS electrophoresis showed that TM was freed from the sediment after heat treatment, showing its high heat stability. Samejima et al. (1985) in a later SDS electrophoresis study reported that TM, TN-I, TN-C from beef myofibrils remained soluble after the heat treatment. Samejima et al. (1982) stated that TM alone did not show any kind of gelation upon heating. The studies by Yasui et al. (1982) on the effects of native tropomyosin agrees with Samejima et al. (1982) that regulatory proteins do not play a role in rigidity of heat-set gels during gelation.

Factors affecting protein gelation

There are many factors affecting heat-induced gelation of meat proteins in meat products and model systems. Factors such as protein concentration, natural condition of the protein, ionic strength of the system, pH of system and thermal processing temperature are among the most important.

Protein concentration

Siegel and Schmidt (1979a) reported that under same extraction conditions, binding ability of crude myosin increased proportionally with protein concentration. However, this was not always true when compared among different extracting procedures. For instance, protein concentrations with a mole ratio of myosin to actin of 2.47 and 3.09 gave a binding strength of 226 g and 303 g, respectively when a Guba-Straub (0.3M KCl, 0.15M K-phosphate, and pH 6.5) extracting solution was used. For a Hasselbach-Schneider (0.6M KCl, 0.1M K-phosphate, 1mM $MgCl_2$, 10mM sodium pyrophosphate, pH 6.4) extracting solution, binding strengths of 310 g and 256 g were obtained for mole ratios of 7.95 and 5.00, respectively. And for Weber-Edsall (0.6M KCl, 0.04M sodium bicarbonate, 0.01M sodium carbonate, pH 9.2) extracting solution, 307 g and 222 g resulted from a mole ratio of 2.21 and 1.31, respectively. They proposed that these results could be attributed to the different ionic environments surrounding myosin molecules.

Acton et al. (1981) found that gel strength increased exponentially with the increase of bovine semitendinosus natural actomyosin concentration at pH 6.0 and 0.6M NaCl in the protein range of 3.75 to 10.0 mg/mL. Gel strength values were 59, 80, 120 and 155 (10^{-3} Newtons) for natural actomyosin concentrations of 4, 6, 8, and 10 mg/mL, respectively. Similar results were found when gel strength values were ploted in a log-log fashion. Samejima et al. (1981,1984), Ishioroshi et al. (1979, 1981), and Yasui et al. (1980) found that gel strength of myosin, myosin heavy chains, rod and subfragment-1 (tested at 65°C, pH 6.0, and 0.6M KCl) increased exponentially by 1.7 to 2.0 (myosin), 0.6 (S-1) and 1.6

(rod) power of the protein concentration depending on ionic strength, pH and presence or absence of actin in the system.

Gel rigidity behavior (rigidity vs M:A plot) was different when F-actin was added to myosin at different myosin:actin weight ratios (M:A). The rigidity of F-actomyosin behaved similar to that of myosin alone in the range M:A of 0.25 to 2.3 (Yasui et al.,.1980). Gel rigidity increased rapidly in the M:A range of 2.3 to 15 (1,250 to 5,500 dyne/cm^2), however, gel rigidity also decreased rapidly to the level of myosin controls (about 2,000 dyne/cm^2) when M:A ratios were increased above 15.

Salt concentration

It has been well known that a portion of myofibrillar protein dissolves and solubilizes when salt is added to meat (Acton et al., 1983; Asghar et al., 1985; Morrissey et al., 1987). Trautman (1964) showed that more salt-soluble protein can be extracted from prerigor than postrigor meat. Gillett et al. (1977) found that mixing salt with ground meat increased the amount of extractable protein. This increment was proportional with salt (NaCl) concentration, with 9-12% the most effective salt concentration for extraction of proteins. Siegel and Schmidt (1979b) determined that the increase of binding properties of crude myosin by salt was due to its ability to solubilize myosin. Salt dissolved myosin by increasing electrostatic repulsions (Hamm, 1960) and by dissociating myosin aggregates (Huxley, 1963). Offer and Trinick (1983) showed the mechanisms of the disruptive effect of sodium chloride on rabbit psoas myofibrils. Salt disrupted structural constraints within the myofibrils, such as Z-lines and M-lines, allowing more myosin and other myofibrillar proteins to be solubilized. They reported that

high concentrations of NaCl (>0.6M) were required to solubilize myosin, actin, C-protein, tropomyosin, troponin, and α-actinin in a more effective way than at lower salt concentrations. The center of the A-band was removed when myofibrils were irrigated with a salt solution of 0.6M, however, most of the A-band is extracted at a salt concentration of 0.8-1.0M. Paterson et al. (1988) studied the effects of sodium chloride by treating isolated beef sternomandibularis muscle myofibrils with various concentrations of NaCl (1.0, 0.7, 0.4, and 0.1M). With the aid of electrophoresis they found that myosin and other myofibrillar proteins were solubilized at high salt concentration in the following fashion: 1.0M>0.7M>0.4M>0.1M. When myofibrils were irrigated with 0.1M or 0.4M NaCl solutions there were no detectable structural changes. In the presence of 0.7M NaCl most of the center of the A-band disappeared.

Macfarlane et al. (1977) found that gel strength of myosin (3.75 and 5.0%), and actomyosin (5.0 and 6.6%) were independent of salt concentration in the range of 0.2 to 0.6M NaCl (that is, up to 3% salt by weight). Binding strength of myosin reached a maximum at 0.2M NaCl (350 g/cm^2) and did not change significantly afterward. Binding strength of 3.7% myosin with maximum NaCl concentration (1.4M) was about 325 g/cm^2. However, they found higher binding values above 0.4M for actomyosin samples. Binding force was 60 g/cm^2 for a 5% actomyosin sample at 0.4M NaCl, and 260 g/cm^2 at 1.4M NaCl. It must be kept in mind that the previous studies described showed the effect of salt on protein solubilization, and that protein solubilization increases proportionally with salt concentration. The result is that gel strength changes directly with salt addition but not because of the higher concentration of salt but because of the high concentration of protein in solution. On the contrary, Macfarlane et al. (1977) had

different salt concentrations but protein concentration was maintained constant. Similar results to Macfarlane et al. (1977) were obtained by Turner et al. (1979). They found an effect of NaCl concentration on binding strength of bullock semitendinosus crude myosin (5% protein concentration) pre- and post-rigor. Binding strength for crude myosin (post-rigor) samples of 5% protein was 210, 250 and 420 g/cm^2 at 0.2, 0.6 and 1.4M NaCl, respectively. For crude myosin (pre-rigor), binding strength was 330, 300 and 490 g/cm^2 at 0.2, 0.6 and 1.4M salt, respectively.

Ishioroshi et al. (1979) found that gel strength of longissimus thoracis rabbit myosin gels did not increase by increasing salt concentration from 0.4-1.0M KCl at a pH 6.0, and that replacement of KCl by NaCl did not affect gel formation. However, there was an effect on gel strength at very low salt concentrations (0.2M) when compared to high concentration (0.6M). SEM studies showed that finer network structures were formed at low ionic strength (0.2M KCl) when compared to those at high ionic strength (0.6M KCl), and higher rigidity values for heat-set gels were associated with gels made of the finer protein network. Rigidity for the heat-set gel at pH 6.0 and ionic strength of 0.2M KCl when heated at 65°C for 25 min was about 7,000 dyne/cm^2, while only 2,000 dyne/cm^2 for an ionic strength of 0.5M KCl. In a later study, Ishioroshi et al. (1983), stated that the higher gel rigidity at low ionic strength developed through the interfilamental head-to-head interaction on the surface of the filaments without involving interaction of the tail portion of myosin molecule, since at low ionic strength (less than 0.3M KCl) myosin forms thick filaments in solution. At high concentration of salt (more than 0.3M KCl) myosin molecules are released from the thick filament of the myofibrils, remaining as monomers in solution.

Hermansson et al. (1986) also found that two types of gel structures can be formed from semimembranosus bovine myosin in the pH range of 5.5-6.0 depending on ionic strength. Heating myosin at pH 5.5, 0.25M KCl at 60°C for 30 minutes caused gelation and the corresponding gel structure was composed of a three-dimensional network of fine strands. When gels were made under the same conditions but at 0.6M KCl a completely different network resulted. This was composed of strands of small globular aggregates. Similar results were obtained when myosin gelled at 60°C for 30 min, pH 6.0 and 0.25M or 0.6M KCl. In both cases the type of structure formed affected gel strength. The finer strands produced at 0.25M KCl had a higher gel strength than the globular aggregates at 0.6M KCl.

pH

Siegel and Schmidt (1979b) did not find any significant effect of pH on binding ability of bovine trapezius crude myosin at 3% protein concentration for binding separate meat pieces. Binding values were 81.1, 92.8 and 80.9 g for pH levels of 6.0, 7.0 and 8.0, respectively. Acton et al. (1981) using a purified natural actomyosin solution, 5 mg/mL in 0.6M NaCl buffer, from bovine semitendinosus muscle found that maximum gel strength occurred at pH 5.0 when studying a pH range of 4.0 to 7.5. Gel strength decreased 2.5 and 2.8 fold in the pH range of 4.0-4.5 and 6.0-7.5, respectively. Gel strength was 70, 90, 200, 170, and 75 (10^{-3} Newtons) at pH 4.0, 4.5, 5.0, 5.5, and remained constant at 6.0-7.5, respectively. The protein network of gels formed at pH 5.0 and 5.5 appeared spongy and syneresis was evident after gel compression. The protein network of gels formed at pH 4.5 and 4.0 were particulate, and for those at pH higher than 6.0 were

uniform and opaque. They obtained similar type of results for crude actomyosin, though gel strength for crude actomyosin systems were higher than those reported for natural actomyosin and the maximum gel strength occurred at pH 5.5 rather than pH 5.0. Acton et al. (1981) addressed the difference in pH optima for gel strength between authors, and they concluded that it might be due to the different methods used to evaluate gel strength rather than the gelation behavior of the myofibrillar proteins. Ishioroshi et al. (1979) found that maximum gel strength occurred at pH 6.0 for purified rabbit longissimus myosin at 4.5 mg/ml in 0.6M KCl at 65°C. Ishloroshi et al. (1979) reported that gel rigidity decreased when pH of the myosin system was either higher or lower than pH 6.0 in the pH range of 5.0 to 8.0. Gel rigidity values were 1,750, 1,150, 570 and 450 dyne/cm^2 for pH values of 6.0, 7.0, 5.0 and 8.0, respectively. Ishioroshi et al. (1981) found similar gel strength behavior of myosin subfragments at 4.5 mg/ml, 0.6M KCl when changing from pH 4.0 to pH 8.0. The maximum rigidity value for HMM (1,000 dyne/cm^2) occurred at pH 5.0, while those for rod (1,000 dyne/cm^2) and LMM (950 dyne/cm^2) occurred at pH 6.0.

Ziegler and Acton (1984) studied protein-protein interactions of bovine natural actomyosin (0.5 mg/mL, 0.6M NaCl) by means of optical density changes when heated from 4°C to 70°C. Solvent pH affected both rate and extent of interactions. The apparent heat of activation (ΔH_a) of 17.1 Kcal/mole at pH 5.5 was lower than activation energies required at pH 6.0 (24.3 Kcal/mole) or pH 7.5 (26.9 Kcal/mole). The lower ΔH_a at pH 5.5 indicated that protein-protein interaction started at a low temperature of 16°C. Since the ΔH_a values were higher above this pH (5.5) protein-protein interactions did not occur until the temperature approached 37°C. Optical density showed maximum protein-protein

interaction at pH 5.5 and 6.0. This results agrees with the findings of Ishioroshi et al. (1979) who reported maximum gel rigidity of heat-set myosin gels at pH 6.0. Yasui et al. (1980) reported that maximum gel rigidity shifted from pH 6.0 to 5.5 when the myosin-to-actin ratio decreased. Acton et al. (1981) found maximum gel strength for natural actomyosin at pH 5.0 to pH 5.5 and for crude actomyosin at pH 5.5. Changes in gel strength might be due to effects by individual sub-unit proteins of myosin and actomyosin molecules. Ziegler and Acton (1984) also found that the two transition temperatures (Tm_1 and Tm_2) of actomyosin, where protein conformational changes occur, changed with pH. Tm_1 of actomyosin at pH 5.5 and at the heating rate of 1°C/min was 43.0°C while at pH 6.0 was 48.5°C. The second transition temperature, Tm_2, changed with pH but to a lesser extent than Tm_1. Tm_2 values were 56°C and 57.5°C for pH 5.5 and 6.0, respectively, a 1.5°C decrease. These decreases on Tm resulted from increasing the H^+ concentration. Goodno and Swenson (1975) found that the increase on H^+ lowered the energy requirements of myosin necessary to produce conformational transitions due to a proton-coupling mechanism.

Temperature

Temperature is another factor that has a tremendous effect on gel structure. It has been reported that in order to form a strong gel from myofibrillar proteins, temperatures must be above 60°C (Ishioroshi et al., 1979; Yasui et al., 1979). Most of the sarcoplasmic proteins coagulate at temperatures between 40°C to 60°C, although some proteins of this group coagulate at temperatures above 60°C. Myoglobin which coagulates at temperature above 65°C (Asghar et al., 1985) is one such protein. Hermansson (1977) defined aggregation in two ways:

1) coagulation as a random aggregation which includes heat denaturation of protein molecules, and 2) gelation as an aggregation during denaturation with formation of continuous protein structures.

Siegel and Schmidt (1979b) found that heating had a linear effect on the binding ability of bovine trapezius crude myosin (5% protein, 3.5% NaCl and pH 6.5) in the temperature range of 45° to 80°C. At lower temperatures (<45°C) the ability of crude myosin to bind pieces of meat was absent. The binding ability was about 30 g at 50°C and reached a maximum of 130 g at 80°C. The binding of meat-pieces is a heat-initiated reaction, since no binding occurs in the raw state (Schmidt and Trout, 1982).

Yasui et al. (1980) studied the effect of temperature on gelation of myosin and actomyosin systems from rabbit leg muscle. Temperature ranged from 20°C-70°C, at pH 6.0 or 7.0, 0.6M KCl and myosin concentration of 4.5 mg/ml. Gel strength of the heat-set gel increased linearly in the temperature range of 20°C-35°C (about 300 dyne/cm^2 for both myosin and actomyosin at 35°C). After this temperature gel rigidity increased in an exponential way until a rigidity maximum was reached at about 60°C (about 1,750 dyne/cm^2 for myosin and 5,000 dyne/cm^2 for actomyosin, M:A of 2.7, at 60°C). No apparent change in gel strength was observed after 60°C. These results agree with later research by Samejima et al., (1981) where myosin and its subfragments showed a similar gel strength development upon gelation. Ishioroshi et al. (1981) also found similar results for the rod part of myosin. Gel strength was about 125 dyne/cm^2 at 35°C and increased to 1,050 dyne /cm^2 at 60°C. Acton et al. (1981) found that gel strength of heat-set natural actomyosin (from bovine semitendinosus) systems with a protein concentration of 7.5 mg/ml in 100mM phosphate buffer, pH 6.0,

with 0.6M NaCl was very discrete. Natural actomyosin gelation occurred at a low temperature of 30°C (Gel strength was 83.5×10^{-3} N) when held for 30 minutes. Gel strength increased by 3.2% (86.2×10^{-3} N) from 30-60°C and by 17.3% from 60°C-80°C (101.2×10^{-3} N). They noted an increase on gel strength when heating after 60°C, this did not agree with previous authors, and it was attributed to actin interaction within the formed gel. It has been shown (Wright et al., 1977) that actin denaturation occurs at a higher temperature than that observed for myosin. Acton et al. (1981) reported a least concentration endpoint value of 6 mg/mL (where the gel remains on the test tube upon inversion) for actomyosin, although actomyosin gels developed at lower protein concentration (as low as 3.75 mg/ml).

Hermansson et al. (1986) did not find any temperature effect on type of gel structure formed. They made bovine semimembranosus myosin gels at 55°C, 60°C, and 65°C in 0.25M or 0.6M KCl at pH 6.0 using a protein concentration range of 7-9 mg/ml. However, they reported some shrinkage and distortion on the strand-like gel structure at increased heating temperatures.

Most studies of protein gelation in meat protein systems have been carried out at constant temperatures in the range of 55°C-70°C. Few studies have been done using different heating rates on proteins systems. It has been suggested (Foegeding et al., 1986), however, that heating rates affect gel rigidity of heat-set gel systems.

Foegeding et al. (1986) studied the effect of heating rates on thermally formed myosin gels (3 and 6 mg/ml myosin). It was found that gelation was dependent on heating rate. Gel strength resulting from the slower rate of 12°C/hr was higher (4.35 and 14.8 mJ at 3 and 6 mg/ml, respectively, final temperature

70°C) than those from 50°C/hr (2.95 and 6.5 mJ at 3 and 6 mg/ml, respectively, final temperature 70°C). They suggested that slow heating rates may allow for more favorable protein-protein interactions. A gel structure was not formed when constant heating (70°C) was applied to myosin at 3 mg/ml. The suspensions finished with a clear upper zone, after cooling, indicating a discontinuous protein phase. However, gelation occurred at 3 mg/ml protein and with slower heating rates. It was suggested that a more random protein-protein interaction was favored by the rapid temperature rise which resulted from the constant heating. Gel formation possibly proceeded according to a nucleation and growth process. More nucleation and less growth would be promoted by faster heating rates. Constant heating temperatures like 70°C would allow more nuclei and less growth to occur. The heat-set gel structure formed also is dependent on protein concentration since at 6 mg/mL a gel developed even when heated at a constant temperature.

PSE condition

Pale, soft, and exudative (PSE) pork is identified by having very light colored, soft and watery lean with an open structure (Briskey, 1964). The development of the PSE condition is associated with an abnormal decrease of muscle pH (5.3-5.4), due to a rapid rate of glycolysis, in the early postmortem period when carcass temperature is still high (Forrest et al., 1975; Pearson, 1987). This condition occurs in stress-susceptible pigs. Approximately 60-70% of stress-susceptible pigs develop PSE musculature within 15-30 minutes after death. Incidence of PSE in ham and loin muscles in United States is about 10% (Topel and Christian, 1986). This condition is caused by muscle protein

denaturation, both sarcoplasmic (Sayre and Briskey, 1963; Scopes, 1964) and myofibrillar (Park et al., 1975; Stabursvik et al., 1984), due to the combination of high lactic acid and and high temperature in the muscle (Briskey and Wismer-Pedersen, 1961). Different methods have been developed to detect stress susceptible pigs before slaughter, and to determine pork quality post mortem. Muscle color and water holding capacity 24 h postmortem (Yang et al., 1984), grading probes (Jones et al., 1984), microscopy (Basrur et al., 1983; Bracchi et al., 1984), electrical conductivity of meat (Pfützner and Rapp, 1988), halothane test response (Eikelenboom and Minkema, 1974; Basrur et al., 1983; McGrath et al., 1984; Topel and Christian, 1986; Webb, 1987) are some of the methods available to measure pork quality.

PSE muscle is inadequate for meat processing (Park et al., 1975). Protein denaturation will cause deleterious economic effects when processing this type of meat due to its color, and poor water holding and bind capacity (Woltersdorf and Troeger, 1989). Color of the longissimus dorsi (loin) will turn pale while ham muscles will exhibit 2-tone appearance, e.g., gluteus medius and biceps femoris will turn pale while gluteus profundus and iliopsoas will retain their normal dark color (Topel et al., 1968). Park et al. (1975) measuring rheological properties of sausage made of PSE muscles found that hardness was much less than those made from normal pork. Hardness for normal sausage at pH 5.69 was 12.0 units while that for PSE sausage at pH 5.44 was 6.4 units. Addition of pyrophosphate greatly improved textural properties of sausage made from normal muscle, whereas it did not improve textural properties of those made from PSE muscle. Hardness for normal sausage at pH 5.67 was 15.7 and 11.45 with and without pyrophosphate, respectively. Hardness for sausage made with PSE muscle at

pH 5.27 was 5.7 and 5.8 with and without pyrophosphate, respectively. These rheological properties of sausage made of normal or PSE muscles changed with ultimate pH. A lower pH resulted in lower values for these properties. Hams made of PSE pork showed a lower cooked yield and poorer sensory quality, with more crumbly and dry texture, when compared to non-PSE ham (Honkavaara, 1988; Maggi and Oddi, 1988). Townsend et al. (1980) studied the effect of PSE meat in fermented dry sausages and found that drying time was shortened up to 50 to 60% compared to normal pork. However, some problems with rancidity development and loss of texture (cohesion and bind) may result. In this study (Townsend et al., 1980) texture of sausages containing only PSE pork was grainy and crumbly and lacked cohesion or bind, resulting in a product having both poor consistency and slicing quality. This was partly due to disruption of the protein filaments that occurs in PSE muscle (Briskey, 1964). Honkavaara (1988) found that when manufacturing dry sausages in a mixture of PSE pork:beef (48:52) there was no textural difference compared to control (all normal pork) dry sausages.

Extractability of myofibrillar proteins is a useful parameter to elucidate protein functionality of the muscle for meat processing. Park et al. (1975) extracted much less myosin B with a Weber-Edsall solution from PSE muscle than from normal muscle. Extractability of myosin B decreased with lowering the pH. Solubility of myosin B was 34.0% and 22.4% for normal muscle at pH 5.78 and 5.44, respectively, while myosin B solubility for PSE muscle was 13.76% and 5.72% at pH 5.42 and 5.26, respectively. Hamm (1960) stated that myofibrillar protein extraction is related to water-holding capacity and binding properties of meat products. The same applies to myofibrillar proteins extracted from PSE

muscle. However, meat products made of PSE muscle show a more marked effect since myosin and the actomyosin complex are already denatured (Penny, 1967a, 1967b, 1969). The extent of this denaturation will depend on how fast the PSE condition developed. In other words, it will depend on how fast the pH drops when body temperature still is high. Penny (1967b) found that extractability of salt-soluble proteins (actomyosin) decreased as much as 50% in PSE muscle when compared to normal muscle. Stabursvik et al. (1984) reported that LMM segment of the myosin molecule was the part which suffered the most extensive denaturation caused by the PSE condition. HMM part of the myosin molecule was left nearly intact. No studies on gelation properties of extracted PSE proteins were found.

Non-meat proteins

Isolated soy proteins and sodium caseinates have been used in meat products for yield improvement and to decrease raw material cost, among many other reasons (Everson, 1984; Sebranek, 1985; Amundson, 1988). However special attention should be given to the effect that these binders will have with respect to the traditional product eating quality characteristics, like texture and flavor (Tuley, 1987). It is important that these proteins be used according to manufacturers instructions in order to maximize their functionality and to result in a product with good quality characteristics.

The effects of these non-meat proteins have been studied in a variety of meat products: emulsified products (Smith et al., 1973; Cassens et al., 1975; Sofos and Allen, 1977; Sofos et al., 1977; Comer, 1979; Comer and Dempster, 1981; Mittal and Usborne, 1985; Amundson, 1986; Coomer et al., 1986; López de

Ogara et al., 1986; Parks and Carpenter, 1987), ground products (Hermansson, 1975; Seideman et al., 1977), hams (Siegel et al. 1979b; Sandoval, 1987), binding of meat pieces (Siegel et al., 1977a, 1979a), model meat systems (Hermansson and Åkesson, 1975) and reviewed in different meat products (Van den Hoven, 1987; Hoogenkamp, 1989).

Soy protein isolates are composed mainly of two protein fractions 7S and 11S (Brooks and Morr, 1985). β-conglycinin is the major 7S globulin and is reported to constitute about 17-21% of the soluble protein fraction. Glycinin, the 11S protein fraction, is made up of about 38-51% of the total protein fraction. However, protein content may vary depending upon the environment, as well genetics and variety of soybean (Murphy and Resurrection, 1984). β-conglycinin is composed of three subunits (α, α', and β) in varying proportions that form six isomers, B_1, B_2, B_3, B_4, B_5 and B_6 (Thanh and Shibasaki, 1978). The general structure of the 7S form of β-conglycinin can be written as $\alpha_x \alpha'_y \beta_z$ where x, y, and z represent the number of the corresponding subunit in the oligomers. β-subunit has higher content of hydrophobic amino acids (alanine, valine, leucine and phenylalanine) than α- and α'-subunits (Thanh and Shibasaki, 1977). Glycinin, on the other hand, is composed of two subunits: acidic and basic. Glycinin contains equal numbers of these two subunits. The structure of the 11S molecule consists of two hexagonal rings of subunits in an alternate arrangement of acidic and basic subunits (Badley et al., 1975). These 12 subunits are packed into two hexagons placed one upon the other to form a hollow oblate cylinder (Moreira et al. 1979). Their amino acid compositions show the basic subunits to be more hydrophobic. The subunits are held together by both hydrophobic and disulfide bonds.

Casein (milk protein) is derived from cow's milk which is composed of approximately 3.5% protein. Casein, a family of related phosphoproteins, comprises about 80% of the total protein content of milk. Casein is composed mainly of 4 different protein fractions: α-, β-, κ-, and a minor component γ-casein (Brunner, 1977). The group of α-caseins (α_{s1}- principal component, and several minor species α_{s0}-,α_{s2}-, α_{s3}, α_{s4}-, α_{s5}-caseins) account for 50-55% of the whole casein. They contain more anionic phosphate groups (8 per mole) than other caseins. β-casein accounts for 30-35% of the casein fraction, while κ- and γ-caseins constitute about 15% and 5%, respectively. The most significant aspects of these caseins are their disproportionate distribution of acidic amino acids, serine phosphate groups and hydrophobic amino acids on their structures (Morr, 1979). This will cause a random coil structure with a low percent helix. α-helix and β-sheet conformations are limited by the high proline content uniformly distributed through the polypeptide chain. Caseins have relatively open flexible structures when compared to globular proteins such as whey proteins (Leman and Kinsella, 1989). α_{s1}-casein has three major hydrophobic regions: amino acid residues 1 to 44, 90 to 113, and 123 to 199. Caseins are very amphiphilic surface-active proteins as a result of this structure. They adsorb rapidly at the surface or interface of liquids and reduce surface and interfacial tension. β-casein's C-terminal region is highly hydrophobic, while the N-terminal has a strong negative charge with 4 of the 5 phosphate groups. Dickinson et al. (1983) found that the more hydrophobic β-casein is more surface active than α_{s1}-casein. Caseinates had surface properties intermediate to β- and α_{s1}-caseins. However, these proteins adsorbed independently and uncompetitively. Caseins exists as micelles (large spherical complexes) in milk. Functionality of caseinates, milk

protein isolates, will vary depending both in the method of manufacture, and in the food system in which they are incorporated. Caseinates have little similarity to the native casein micelles in milk. However, caseinates (sodium and potassium) still have good functional properties. They are highly soluble in water, can be dispersed rapidly in aqueous mixtures, possess the ability to form interfacial films, and exhibit resistance to thermal denaturation and/or coagulation, which means that the protein film remains relatively stable over a wide range of pH, temperature, and salt concentrations.

Gelation mechanisms responsible for evaluating many quality effects are difficult to study in intact meat mixtures. Most gelation studies have utilized simple purified protein solutions without consideration of the complex interaction of the mixture of muscle proteins. The same has been true for meat protein systems and for meat protein systems with non-meat protein addition.

Peng et al. (1982a, 1982b) studied the interaction of 11S protein subunits and myosin (from rabbit leg and back muscles). It was found that there were no interactions between soy 11S and myosin when heated at 70°C. When temperature reached 85°C, 11S protein started to dissociate and the acidic subunits remained soluble after dissociation. Basic subunits became insoluble and precipitated. However, basic subunits were the ones reported to interact with myosin heavy chains when heated in the range 85°-100°C. Results also showed that this interaction did not occur between intact, undissociated myosin and 11S molecules, but rather, between dissociated myosin (MHC) and partially or fully dissociated 11S protein (basic subunits). Hydrophobic bonding interactions between 11S basic fraction and myosin (MHC) were reported to be involved. The experimental conditions used were 1.14 mg/ml of each protein, pH 6.5, 0.4M

NaCl, 58.3mM Na-PO4, $\mu=0.54$, heat treatment of 75°, 85°, 95° and 100°C for 30 minutes.

Haga and Ohashi (1984) found that upon heating in a stepwise fashion, first at 70°C for 4 min and then at 100°C for 30 min, the denatured 11S (cold insoluble fraction) protein associated continuously around the myosin B (actomyosin) network reinforcing it. The protein network of this mixture was more elaborate than those of myosin B alone. It was reported than even before heat treatment the mixed solutions formed a rough network. They suggested that the interactions between these two proteins resulted from disulfide bonds formed before heat treatment. This findings differ from those from Peng et al. (1982a, 1982b). The reason might be a different protein concentration. Haga and Ohashi (1984) used experimental conditions of 15 mg/ml myosin B (from longissimus thoracis and hind legs of rabbits), 50 mg/ml 11S protein, 0.6M KCl, pH 7.0 and 50 mM phosphate. There were no data presented to suggest which subunits were involved in the protein network formation.

Utsumi and Kinsella (1985) found that β-subunit of the 7S globulin had a high affinity for the basic subunit from the 11S globulin, and in this way could interact and form part of the gel matrix. Contribution of acidic subunits to the protein network structure was limited in soy isolates. Soy protein solutions used for making the gels were 12% and 15%, w/v, in 30mM Tris-HCl buffer (pH 8.0) and they were heated at 80°C for 30 min. The authors reported that electrostatic interactions and disulfide bonds were involved in the formation of 11S globulin gels, while hydrogen bonds were important in gelation of 7S globulin. In the case of gels made of isolated soy proteins, the contribution of hydrophobic interactions apparently were higher than in 7S and 11S globulin gels, but

hydrogen bonds also were involved. They studied the effect of salt on gelation of 7S, 11S, and soy isolate. Hardness decreased for 11S globulin gels and for soy isolates gels when salt concentration was increased from 0.1M to 0.5M NaCl. This may be due partly to the stabilizing effect of NaCl on thermally induced changes in their structures (Damodaran and Kinsella, 1981). This stability can be visualized as an inhibition of dissociation of these proteins into subunits (Kilara and Sharkasi, 1986). NaCl did not show any effect on 7S gelation, indicating limited ionic interactions (Babajimopoulos et al. 1983).

King (1977) reported the formation of a complex between rabbit myosin heavy chains and β-conglycinin subunits when they were heated at high temperature, 75°C to 100°C, in a phosphate buffer (pH 7.6, NaCl 0.4M, 10 mg/ml myosin and 10 mg/ml 7S). King did not report which type of subunits were involved in gelation with myosin. Peng and Nielsen (1986) found that interactions occurred between 7S (2 mg/ml) and myosin (2 mg/ml) at temperatures of 50°C to 100°C. However, 7S interfered with the self aggregation of chicken myosin heavy chains by forming a complex of 7S and MHC which remained in the supernatant rather than forming part of the structural protein network. The protein ratio of 7S to MHC was 1:1, salt concentration was 0.4M NaCl and pH 6.5.

Explanation of Thesis Format

The dissertation is divided into three parts, each part being a complete paper to be submitted to a scientific journal. Part I examines the effect of heating rate and protein concentration on gel strength and water loss of muscle protein

gels. This paper has been published (Journal of Food Science 54:850, 1989). Part II examines gelation characteristics of muscle proteins from PSE pork. Part III examines the effect of non-meat proteins on gelation properties of porcine muscle proteins. The research was conducted by the candidate under the guidance of his major professor.

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PART I. EFFECT OF HEATING RATE AND PROTEIN CONCENTRATION ON GEL STRENGTH AND WATER LOSS OF MUSCLE PROTEIN GELS

EFFECT OF HEATING RATE AND PROTEIN CONCENTRATION ON GEL STRENGTH AND WATER LOSS OF MUSCLE PROTEIN GELS

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ABSTRACT

The effects of linearly increasing heating rates (17, 38 and 85°C/hr) and protein concentration (10, 20, 30, 40 and 50 mg/mL) on thermally induced gels made of extracted salt-soluble proteins were evaluated. Gel strength analyses indicated that the force needed to compress muscle protein gels decreased when heating rate was increased. A greater water loss from compressed gels occurred at protein concentrations of 10 and 20 mg/mL than at 30 to 50 mg/mL. Above 30 mg/mL, water loss tended to be relatively constant. Protein loss in the expelled water, after compression, was less for the slower heating rate, and larger as heating rate increased. The total amount of protein in the expelled water increased with increasing protein concentration in the system. SDS gel electrophoresis demonstrated a change in some of the expelled proteins at different heating rates.

INTRODUCTION

Most studies conducted on rheological properties of extracted muscle protein gels have investigated myosin, actomyosin and their combination with other myofibrillar protein fractions. Blood plasma proteins also have been studied as have soy proteins and milk proteins. The effects of factors such as pH, salt, protein concentration and heating temperatures on these protein gels have been studied also (Nakayama and Sato, 1971; Macfarlane et al., 1977; Ishioroshi et al., 1979; Siegel and Schmidt, 1979a,b; Acton et al., 1981, 1983; Hermansson, 1982a,b, 1985, 1986; Asghar et al., 1985; Hermansson et al., 1986; Hoogenkamp, 1986; Samejima et al., 1986). It has been found that myosin or actomyosin in the system largely determines the quality and strength of binding in sausages and in model systems. Many of these studies were conducted with low-protein-concentration systems (below 10 mg/mL) and at constant heating temperatures. Salt concentrations and pH in these studies have been varied from ranges of well below to levels above conditions characteristic of practical meat processing operations.

Little information is available on textural properties of extracted muscle protein gels at higher protein concentration (>10 mg/mL) and use of increasing heating rates instead of constant heating temperatures. It has been suggested that slow heating rates may allow more protein-protein interactions to occur and form a more ordered and stronger 3-dimensional gel. Foegeding et al. (1986a) evaluated the importance of using different heating rates on the properties of thermally induced myosin, fibrinogen and albumin gels. Also, Foegeding et al. (1986b) investigated gelation properties of mixtures of these proteins.

The objective of this study was to investigate the effect of heating rates on quality characteristics of thermally formed protein gels that closely resemble processed meat products. The studied proteins included numerous sarcoplasmic proteins as well as other muscle constituents in addition to myofibrillar proteins, all of which may contribute to or detract from 3- dimensional gel formation. Water retention by gels formed at different heating rates also was evaluated to assess the potential for yield effects during heating of processed meats. Finally, SDS gel electrophoresis was utilized to confirm the protein composition of the extracts and to study the proteins excluded from the gel and contained in the expelled water resulting from gel compression.

MATERIALS AND METHODS

Protein Extraction and Preparation

Semimembranosus muscles from market-weight barrows were obtained from the Meat Laboratory, Iowa State University, within 96 hr of slaughter. Samples were kept under refrigeration at 0-1°C until further use, no more than 12 hr.

Muscle samples were ground through a 4.5-mm plate, weighed and homogenized (Vita-mix 3600, Cleveland, OH) using 1 part of meat with 2.2 parts of a 1°C solution containing 0.56M NaCl, 17.8mM $Na_5P_3O_{10}$, pH 8.3, and 1mM NaN_3 for 30 sec. This solution corresponds to 2.5% salt and 0.5% sodium tripolyphosphate, similar to levels used in commercial processed meat products. The mixture was placed In a cold room at 1°C for 1 hr to extract salt-soluble protein. The mixture then was centrifuged (Beckman Model J-21C Centrifuge, Palo Alto, CA) at 12,000 x g, 2°C for 1 hr, and the protein extract separated from the residue and strained through gauze. This protein extract was kept overnight at 1°C until further use. The extract was examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein characteristics. Protein concentration of samples was adjusted by diluting the initial protein extract to the desired concentration range (10 to 50 mg/mL) with a salt-phosphate solution of the same ionic strength as the original.

The meat pH was determined by mixing 10 g ground muscle with 100 mL of deionized-distilled water for 5 min and measuring the slurry with a Fisher Accumet® selective ion analyzer (Model 750, E-5D pH electrode).

Soluble Protein

The protein concentration of the extracted samples and their dilutions were measured by the Biuret method (Gornall et al., 1949). Biuret reagent, 5 mL, was added to 1 mL of extracted protein, and the combination was mixed on a Vortex® and allowed to stand for 15 min to attain maximum color development. Absorbance was read on a Hach DR/3000 spectrophotometer (Loveland, CO) at 540 nm and converted to mg protein per mL of solution by using a Bovine Serum Albumin (BSA) standard curve. Protein concentration of the samples was recorded as mg of soluble protein per mL of solution.

SDS-Gel Electrophoresis

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on protein extracts and proteins in the expelled water from heat-induced gels by using a modification of Laemmli (1970) with 8% acrylamide [bis-acrylamide/acrylamide, 1:30 (w/v)] slab gels (pH 8.9) of 14 x 12 x 0.15 cm dimension. Protein samples were incubated at 50°C for 20 min in 7.5% (v/v) 2-mercaptoethanol, 1.5% (w/v) SDS, 15% (w/v) sucrose, 0.3% (w/v) bromophenol blue, 15mM 2-N-morpholinoethanesulfonic acid (pH 6.5). The amount of protein loaded on the polyacrylamyde gel ranged from 35 to 164 µg in a load volume of 35 µL. Electrophoresis was conducted at room temperature with 30 mA current per gel for about 4.5 hr using Tris-glycine buffer (pH 8.6) as electrode buffer. Gels were stained in 0.2% Coomassie brilliant blue, 40% methyl alcohol, 7% acetic acid for 3-4 hr and destained in 40% methyl alcohol, 7% acetic acid for 5 hr. Molecular weights were determined by comparing mobilities of proteins in the

samples with mobilities of a high molecular weight standard mixture (Sigma, St. Louis, MO).

Heat Treatment

Aliquots (30 g) of the protein extract were placed in 100 mL beakers. Aluminum foil was placed on top of the beakers to avoid evaporation during heating. The samples were allowed to equilibrate at room temperature for 30 min before heat treatment. Temperature was monitored by using an Omega digital thermometer equipped with multiple thermocouples. Both the water bath and sample temperatures were recorded. The temperature of the water bath was continuously regulated to give linearly increasing rates in the sample of 17, 38 or 85°C/hr. In all cases, when the sample reached 70°C, the heating was stopped. The beakers were removed and placed in a container with ice, which was placed in a cooler at 1°C overnight. Samples were run in triplicates, and each treatment had three replications.

Gel Strength

Gel strength was evaluated by the compression-extrusion method (Bourne, 1982) sometimes referred to as back extrusion (Acton et al., 1981) or annular pump (Harper et al., 1978; Steffe and Osorio, 1987). An Instron (Model 1122) Testing device was used and gel strength was measured by using a plunger 35 mm in diameter and compressing the protein gel twice, in a 45 mm diameter beaker, at a constant speed of 100 mm/min to a final 20 % of its original height. The highest peak of the first compression curve gave the force needed to compress the protein gel (80 % compression), and gel strength was recorded in kg.

Water Loss

After the gels were compressed, they were transferred to a 25x100 mm tube and centrifuged at a low speed of 476 x g for 10 min. Centrifugation accomplished the separation of water expelled from the surfaces of the gel by compression from the Instron. Low-speed centrifugation was used to minimize structural breakdown from centrifugal forces. The residue was weighed and the liquid loss expressed as the difference in weight from the initial 30 g sample.

Protein Loss

A 1 mL aliquot of the liquid expelled from the gels and separated by centrifugation was taken, and protein concentration was measured by the Biuret method. The expelled liquid also was examined, using SDS-PAGE, for characteristics of the proteins contained in the liquid phase.

Statistical Analysis

The experiment was replicated three times and results were analyzed using a 3x5 factorial design with three heating rates and five protein concentrations. The Statistical Analysis System (SAS, 1986) was used to determine means, standard errors and analysis of variance. Least significant difference ($P<0.05$) was used as a method of means separation.

RESULTS AND DISCUSSION

Gel Strength

The strength, measured by the first compression, of heat-induced gels of salt-soluble proteins, containing both sarcoplasmic and myofibrillar fractions, as a function of protein concentration and heating rate is shown in Fig. 1. There was a highly significant difference (P<0.01) due to protein concentration. Gel strength was directly proportional to the amount of protein in the heated solution. Gel strength increased in a log-log fashion, with the increase of salt-soluble protein concentration from 10 to 50 mg/mL. This is similar to that of Acton et al. (1981) who found that gel strengths for natural actomyosin gels at pH 6.0 increased exponentially with a protein concentration range of 3.75 to 10.0 mg/mL. The pH of muscle samples ranged from 5.5 to 5.7, and the pH of protein extracts in our study were 6.1. Ishioroshi et al. (1979), using a myosin concentration range of 1 to 10 mg/mL, also found a log-log relationship of the shear modulus of the gels with protein concentration.

Gels formed at a concentration of 10 mg/mL in this study were very soft, but they remained in the beaker upon its inversion. This is similar to the least concentration end-point value of 6 mg/mL for natural actomyosin reported by Acton et al. (1981). Diameter of the containers where gels were formed in this study were 45-mm beakers. Ten-mm tubes were used by Acton et al. (1981).

Gel strength was measured by the Instron Texture Profile Analysis (TPA) as hardness or the peak force during the first compression cycle (Bourne, 1978). Cohesiveness is the ratio of the positive force area of the second compression to that of the first compression. Montejano et al. (1985) found that stress and TPA

hardness, and strain and TPA cohesiveness correlate strongly with each other. The significant effect of protein concentration on gel strength, in this study, supports the findings of Hamann (1987) as well as others that hardness of processed muscle foods is strongly influenced by protein concentration.

Heating rate had a significant effect (P<0.01) on the protein gel strength of these extracts. At all protein concentrations, the lowest heating rate resulted in the greatest gel strength, whereas the fastest heating rate resulted in the weakest gel strength. The force needed to compress the protein gels (in the range 10 to 50 mg/mL protein) ranged from 0.17 to 7.72 kg for the 17°C/hr heating rate, 0.14 to 6.53 kg for the 38°C/hr, and 0.08 to 5.97 kg for the 85°C/hr. Other workers (Foegeding et al., 1986a) have reported similar results but at considerably lower (3-6 mg/mL) protein levels. These findings also are supported in a recent report by Saliba et al. (1987) in which the shear stress of frankfurters were related to the same heating rate pattern.

In all instances In the present study, the fastest heating rate of 85°C/hr allowed for a gel structure to develop. At 10 mg/mL, the developed gel was very soft, but there was no clear upper zone indicating a discontinuous protein phase or gel separation. At this concentration, there was enough protein to achieve protein-protein interactions and gel formation at all heating rates studied.

Structural damage to gels from compression can be estimated if the second-compression value is expressed as a percentage of the value obtained during the first-compression cycle (Foegeding, 1987). The highest peak of the second-compression curve also was measured in this study (Fig.2). This shows that gel strength of the second-compression cycle also was directly proportional to protein concentration, as it was for the first-compression cycle. Although the

structure of the gels has already failed or broken after the first compression, the graph shows that the failed gel structure still retains some structural integrity, but the damage to the original gel structure was severe. The force needed to compress the gel for the second time ranged from 24 to 42% of that needed for the first compression. Foegeding (1987) found that second-cycle hardness values were 36 to 45% the force of the first-cycle hardness for turkey salt-soluble proteins. The heating rate effect on the second compression is different than on the first compression. The 17°C/hr rate resulted in significantly higher (P<0.05) gel strength at 40 and 50 mg/mL of protein when compared to the 38°C/hr, but was not significantly different (P>0.05) than the 85°C/hr.

Water Loss

Water losses were examined with the hypothesis that gel formation might explain yield changes (water losses) that occur when processed meat products are exposed to different heating regimes. There was an effect of protein concentration on water loss as shown in Fig. 3. Greater water loss occurred at protein concentrations of 10 and 20 mg/mL compared with 30 to 50 mg/mL. Above 30 mg/mL, water loss tended to be relatively constant (around 370 mg/g of gel). The largest amount of water loss occurred at the lowest protein concentration, indicating that, not only was the available protein in the matrix not enough to make a strong gel, but also was not enough to effectively trap water.

At the low protein concentration (10 mg/mL), the slowest heating rate (17°C/hr) resulted in water loss of about 670mg/g of gel. Although water loss for the other two heating rates were measurably lower than at 17°C/hr, it is likely that heating rate did not have a real effect on water loss at these faster rates. The

gels formed at 10 mg/mL for both 38 and 85°C/hr heating rates were very soft and appeared visually to be something less than a true gel. At the same time, this "slurry" or "solution" retained more water when centrifuged at low speed than did the more typical gel that was formed at the 17°C/hr rate. After centrifugation of the two samples heated at faster rates, a small amount of water was removed but a jelly-like sediment was retained, which included a considerable amount of water. This sediment was found after centrifugation to continuously show a slow separation of additional water. For samples of high protein concentration (30-50 mg/mL), a more structured gel resulted in a solid sediment after centrifugation. It was observed that these sediments from the higher-protein gels also continued to lose some water after centrifugation but at a much lower rate than at low protein concentrations. Thus, the low-speed centrifugation used in this study to measure free or separable water in the gel did not always reflect the weak gel strength that occurred at low protein concentration.

There was no relationship between gel strength and water retention in this study. Hermansson and Lucisano (1982) pointed out that, when evaluating protein gels, waterbinding and texture should be treated separately because they are two different gel properties.

Heating rate did not effect water loss from the gels because at all protein concentrations, with the exception of 10 mg/mL, there was no significant difference in amount of water lost ($P > 0.05$).

Protein Loss

Protein loss after compression as a function of protein concentration and heating rate is shown in Fig. 4. There was a highly significant difference ($P < 0.01$)

due to the effect of protein concentration. A higher protein concentration resulted in greater protein loss in the expressed juice after gel compression.

Heating rate also had a significant effect on protein loss. At all protein concentrations, protein loss was less for the slower heating rate and greater as heating rate was Increased. Protein-protein interactions may be more complete at a slower heating rate, resulting in a more complete, extensive 3-dimensional gel structure of the protein molecules, thus contributing to both increased gel strength and less protein loss in the expelled water.

SDS Electrophoresis

The SDS-PAGE patterns of a high molecular weight standard mixture (lane 1), extracted-salt-soluble proteins containing both sarcoplasmic and myofibrillar proteins (lanes 2-5), and the protein profile carried in the expelled water from heat-induced gels after compression (lanes 6-14) are shown in Fig. 5. The reasons for using this electrophoretic technique in the present study were: 1) to monitor the extracted-salt-soluble-protein solution to insure the same type and ratio of myofibrillar and sarcoplasmic protien fractions, and 2) to check the expelled water for selective separation of proteins during the formation of the gel structure.

There was no dilution effect on protein profiles of the extracted-salt-soluble protein as shown by lanes 2 to 5, where the extracted-protein concentrations were 50, 40, 30 and 20 mg/mL, respectively. Myosin was the major protein in these fractions.

Proteins that were not bound to the gel network were released in the exuded water after compression of the gel (lanes 6 to 14). It appears that there is a

selection of proteins that occurs during formation of a structured gel. The relative intensity of protein bands supports this idea. Protein fractions A (which may be porcine albumin) and B showed the same relative degree of fading as the protein system was diluted (lanes 6 to 14). This may indicate that these proteins are involved in the formation of the gel structure at all concentrations studied. Band intensities for fractions C (tropomyosin) and fraction D appeared to be relatively constant, independent of total protein concentration. This may suggest that these polypeptides are not used in the gel structure resulting in their expulsion in the exuded water. This is in accordance with Samejima et al. (1982) who found that tropomyosin did not participate in gel formation because of its high-heat stability. Shiga et al. (1988) also found that tropomyosin from ground chicken muscle was extracted easily from a gel heated at 70°C. In the case of the fraction E polypeptide, the protein band intensity increased when the protein content in the system was diluted. Finally, there appeared to be a heating rate effect on fraction F. At the lowest heating rate (17°C/hr) the band appeared to be lighter than at the two faster heating rates, being the most intense at 85°C/hr. This implies that at lower heating rates this polypeptide may interact in a more extensive way with the other proteins in the gel for making the gel network firmer than at higher heating rates.

This electrophoretic technique shows the effects of protein concentration and heating rates on protein profiles in the expelled water from heat-induced gels. The results suggest a selective interaction between muscle proteins during gel formation. However, a more in-depth study is needed to better understand how these factors affect the complexity of protein interaction during the formation of a 3-dimensional-gel structure.

CONCLUSIONS

Compression studies of salt-soluble protein gels containing both sarcoplasmic and myofibrillar fractions showed that heating rate significantly affects gel strength and protein loss after compression. The effects of protein concentrations on gel strength also were significant as previously reported by other researchers.

At all protein concentrations, the slowest heating rate resulted in the greatest gel strength, and gel strength decreased with increasing heating rate. Results also showed that the slowest heating rate resulted in lower protein loss and that increasing heating rates resulted in an increase of protein in the liquid exuded after compression. This also may suggest that protein gel formation is more complete at slower heating rates.

Heating rate did not have any significant effect on total water loss from the gels. However, protein concentration above 30 mg/mL resulted in a constant water loss, whereas below 30 mg/mL, considerably greater water loss occurred.

SDS gel electrophoresis showed that heating rates also influenced the relative amounts of some proteins in the expelled water from the gel suggesting that heating rate plays a role in selective interaction between specific proteins during gel formation.

This study demonstrated that protein mixtures (sarcoplasmic and myofibrillar) could be controlled in terms of hardness characteristics by heating rates during the gel formation. This suggested that processed meat texture and firmness could be affected by heating rates. It is reasonable to assume that an "ideal" heating rate for maximum product yield and textural properties should be attainable if the gelation mechanism is well understood. Additional work is

needed, however, on the effects of nonmeat proteins and protein ratios on gelation properties of meat proteins and finished product characteristics of meat mixtures. More work is needed also on the relationship between protein gelation and water retention characteristics of meat protein gels, particularly at various heating rates.

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Figure 1.   Gel strength (first compression curve) of heat induced gels from
extracted salt soluble muscle proteins. [The standard errors of the means
for the measurements at protein concentrations of 10, 20, 30, 40 and 50
mg/mL were 0.005, 0.011, 0.075, 0.327 and 0.197, respectively.]
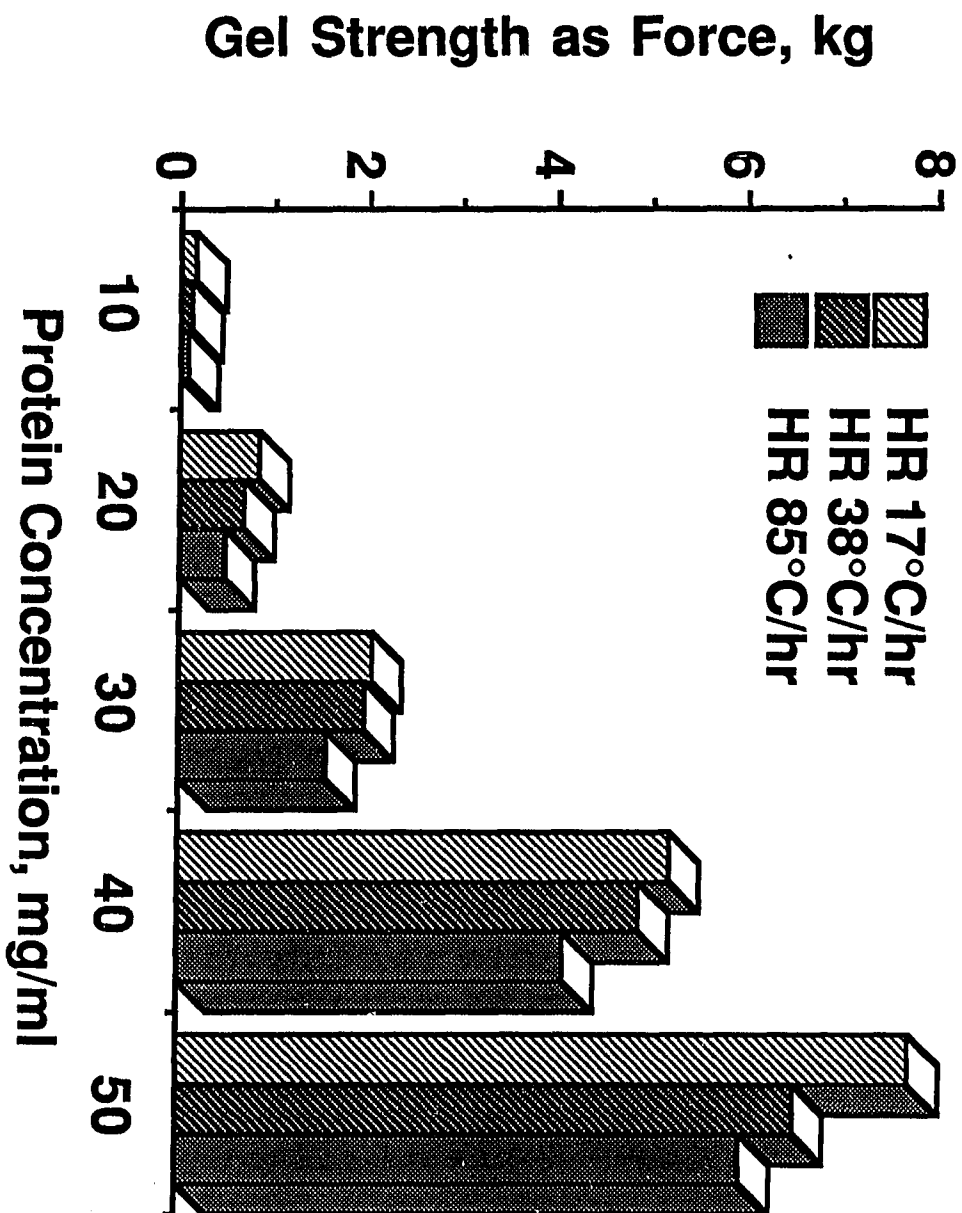
Gel Strength as Force, kg

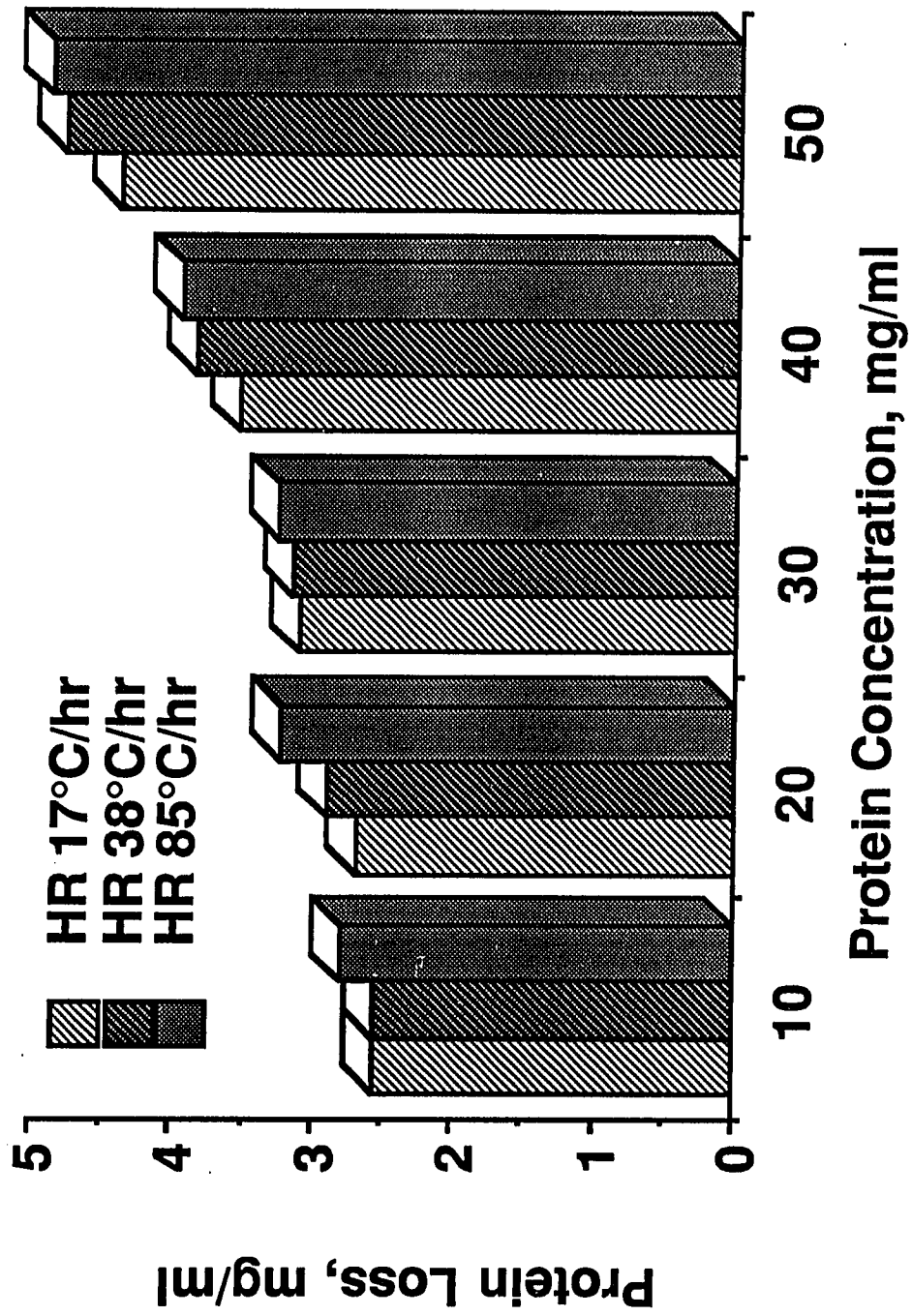Protein Concentration, mg/ml

HR 17°C/hr
HR 38°C/hr
HR 85°C/hr

Figure 2.   Gel strength (second compression curve) of heat induced gels from extracted salt soluble muscle proteins. [The standard errors of the means for the measurements at protein concentrations of 10, 20, 30, 40 and 50 mg/mL were 0.004, 0.034, 0.141, 0.075 and 0.079, respectively.]
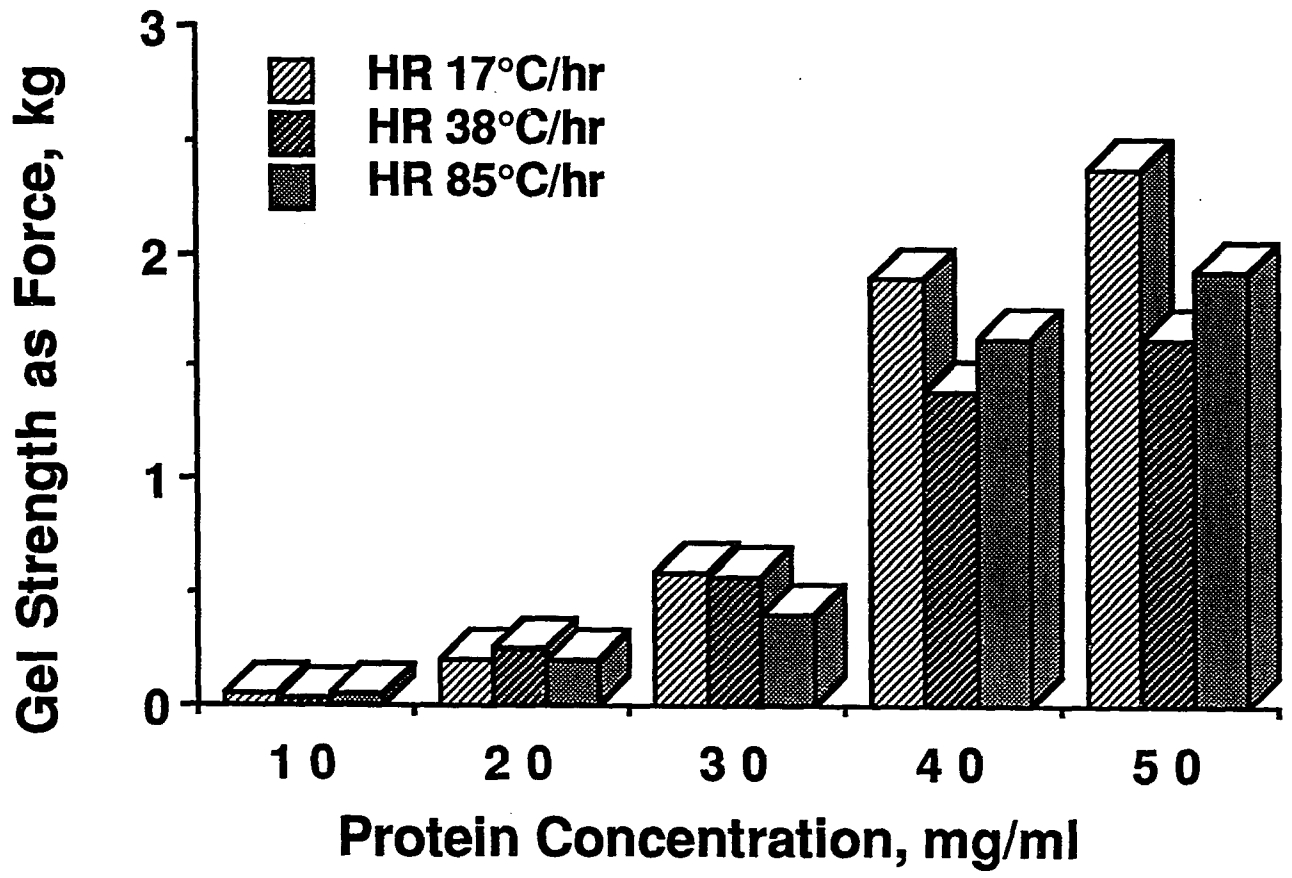
Figure 3.   Water loss from gels after compression. [The standard errors of the means

for the measurements at protein concentrations of 10, 20, 30, 40 and 50

mg/mL were 0.735, 0.651, 0.31, 0.16 and 0.12, respectively.]

Figure 4.   Protein content of expelled liquid after gel compression. [The standard
errors of the means for the measurements at protein concentrations of 10,
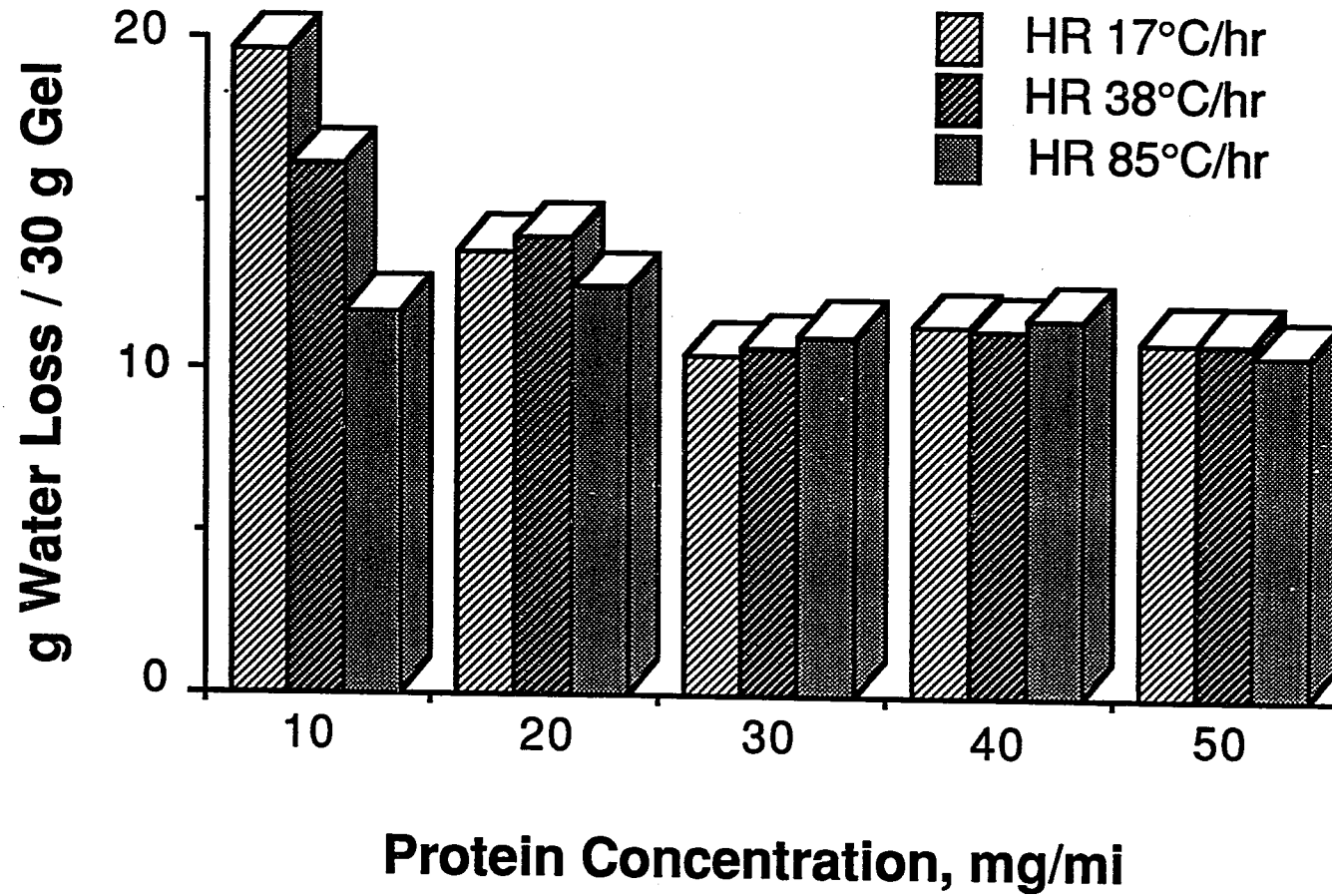20, 30, 40 and 50 mg/mL were 0.015, 0.022, 0.029, 0.012 and 0.015,
respectively.]

Figure 5.   SDS polyacrylamide gel of extracted-salt-soluble protein (sarcoplasmic

and myofibrillar protein) and soluble protein in the exuded water from

heated gels after compression. [Thirty-five µL of protein solution were

loaded in each well. Lane 1 contains standard protein markers of 29 to 205

Kilodaltons. Lanes 2,3, 4 and 5 correspond to salt-soluble-protein extracts

of 50, 40, 30 and 20 mg/mL. Lanes 6,7 and 8 show soluble protein in the

released water when heating rates were 17, 38 and 85°C/hr respectively,

and a protein solution of 50 mg/mL. Lanes 9, 10 and 11 show the

respective heating rates at 30 mg/mL protein, and lanes 12, 13 and 14

show the same heating rates at 20 mg/mL protein. The amount of total

protein loaded in each well was as follows lane (µg): 1(35), 2(35), 3(35),

4(35), 5(35), 6(143), 7(150), 8(164), 9(101), 10(108), 11(119), 12(87),

13(98) and 14(108).]
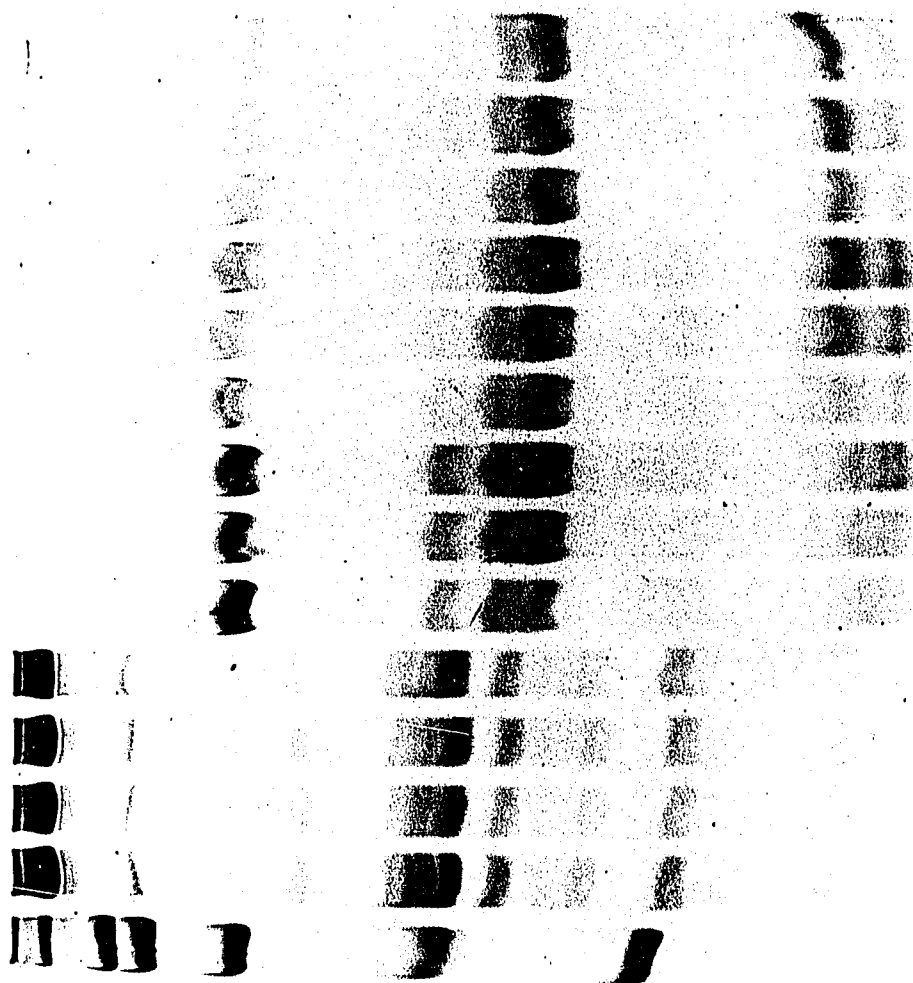
PART II. GELATION CHARACTERISTICS OF MUSCLE PROTEINS FROM PSE PORK

GELATION CHARACTERISTICS OF MUSCLE PROTEINS
FROM PSE PORK

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ABSTRACT

Thermally induced protein gels were made using extracted salt-soluble proteins from normal pigs and stress susceptible pigs determined to be PSE. Effects of heating rates (17, 39 and 93°C/hr) at various protein concentrations (23, 34, 48 and 54 mg/ml) were evaluated. Gel strength of PSE extracts was 45% of the controls at equivalent protein concentration. Gel strength of normal and PSE-muscle protein gels from the first compression curve increased with increasing protein concentrations at all heating rates; however, gel strength was greater for slow heating rates than for fast heating rates in both PSE and normal samples. Percent water loss was greater for PSE extracts than for controls at the same protein concentration. Losses of 47% and 36% for PSE and controls, respectively, were observed at a protein level of 54 mg/ml protein. There was no heating rate effect on water losses in either case. Protein loss was lower, for both PSE and control, at low protein concentrations than at high protein content in the range studied. Slow heating rates resulted in less protein loss, for both PSE and control, while faster heating rates gave higher protein losses in the exuded water.

INTRODUCTION

The physical-chemical state of myofibrillar proteins is very important in meat processing, since the proteins largely confer functionality to the meat system. Myosin and actomyosin complex are the principal myofibrillar proteins in prerigor and in postrigor meats that show a close relationship with the binding quality of comminuted meat products (Yasui et al., 1982). Any circumstance that may alter this state could dramatically affect myofibrillar protein functionality. Pale, soft, exudative pork (PSE) is a classical example of poor protein functionality as a result of chemical changes that occur in the muscle early postmortem (Forrest et al., 1975). PSE muscle has been reported to result in muscle protein denaturation due to the combination of high muscle temperature and rapid lactic acid production immediately post mortem (Penny, 1969; Park et al., 1975; Topel and Christian, 1986).

Many methods have been developed to detect stress susceptible pigs before slaughter, and to determine pork quality post mortem. Muscle color and water holding capacity 24 h post mortem (Yang et al., 1984), grading probes (Jones et al., 1984), microscopy (Basrur et al., 1983; Bracchi et al., 1984), and electrical conductivity of meat (Pfützner and Rapp 1988) are some of the methods available to measure pork quality. Halothane test response is a method that has been used to detect stress susceptible pigs before slaughter (Basrur et al., 1983; Topel and Christian, 1986; Webb, 1987).

The objective of this study was to investigate the quality characteristics of thermally formed protein gels from PSE pork and to determine whether processing treatments could be used to compensate for the reduced functionality

of PSE pork proteins. The extracted salt-soluble protein mixture, in this study, included both sarcoplasmic and myofibrillar fractions that closely resemble the extracted fraction that occurs in processed meat products. In addition, the effects of heating rates on water retention by gels were evaluated to assess the potential for yield effects during heating of processed meats. Finally, SDS gel electrophoresis was utilized to look for protein differences in the salt-soluble-protein extracts and in the exuded water of heat-set gels from normal and PSE pigs. The protein profiles obtained were examined for specific interactions between proteins of the salt-soluble extracts as a result of heat treatment.

MATERIALS AND METHODS

Protein Extraction and Preparation

Semimembranosus muscles from halothane positive and halothane negative barrows were obtained from the Meat Laboratory, Iowa State University, within 96 hr of slaughter. Samples were kept under refrigeration at 0-1°C until further use, no more than 12 hr.

Muscle samples were ground through a 4.5-mm plate, weighed and homogenized (Vita-mix 3600, Cleveland, OH) using 1 part of muscle with 2.2 parts of a 1°C solution containing 0.56M NaCl, 17.8mM $Na_5P_3O_{10}$, pH 8.3, and 1mM NaN_3 for 30 sec. This solution corresponds to 2.5% salt and 0.5% sodium tripolyphosphate, similar to levels used in commercial processed meat products. The mixture was placed in a cold room at 1°C for 1 hr to extract soluble protein. The mixture then was centrifuged (Beckman Model J-21C Centrifuge, Palo Alto, CA) at 12,000 x g, 2°C for 1 hr, and the protein extract separated from the residue and strained through gauze. This protein extract was kept overnight at 1°C until further use. The extract was examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein characteristics. Protein concentration of samples was adjusted by diluting the initial protein extract to the desired concentration range (23 to 54 mg/mL) with a salt-phosphate solution of the same ionic strength as the original.

The meat pH was determined by mixing 10 g ground muscle with 100 mL of deionized-distilled water for 5 min and measuring the slurry with a Fisher Accumet® selective ion analyzer (Model 750, E-5D pH electrode).

Soluble Protein

The protein concentration of the extracted samples and their dilutions were measured by the Biuret method (Gornall et al., 1949). Protein concentration of the samples was recorded as mg of soluble protein per mL of solution.

SDS-Gel Electrophoresis

A discontinuous sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on protein extracts and proteins in the expelled water from heat-induced gels by using a modification of Laemmli (1970) with 4% acrylamide [bis-acrylamide/acrylamide, 1:30 (w/v)] stacking gel (pH 6.9) and 10% acrylamide [bis-acrylamide/acrylamide, 1:30 (w/v)] resolving gel (pH 8.9) of 14 x 12 x 0.15 cm dimension. Protein samples were incubated at 50°C for 20 min in 7.5% (v/v) 2-mercaptoethanol, 1.5% (w/v) SDS, 15% (w/v) sucrose, 0.3% (w/v) bromophenol blue, 15mM 2-N-morpholinoethanesulfonic acid (pH 6.5). The amount of protein loaded on the polyacrylamyde gel ranged from 50 to 141 µg for normal pigs and 40 to 146 µg for PSE pigs in a load volume of 40 µL. Electrophoresis was conducted at room temperature with 30 mA current per gel for 4.5 hr using Tris-glycine buffer (pH 8.6) as electrode buffer. Gels were stained in 0.2% Coomassie brilliant blue, 40% methyl alcohol, 7% acetic acid for 3-4 hr and destained in 40% methyl alcohol, 7% acetic acid for 5 hr. Molecular weights were estimated by comparing mobilities of proteins in the samples with mobilities of a high molecular weight standard mixture (Sigma, St. Louis, MO). This mixture included rabbit myosin (205K), ß-galactosidase (116K), phosphorylase B (97.4K), bovine albumin (66K), egg albumin (45K) and carbonic anhydrase (29K).

Heat Treatment

Aliquots (30 g) of the protein extract were placed in 100 mL beakers. Aluminum foil was placed on top of the beakers to avoid evaporation during heating. The samples were allowed to equilibrate at room temperature for 30 min before heat treatment by submersion in a controlled temperature water bath. Temperature was monitored by using an Omega digital thermometer equipped with multiple thermocouples. Both the water bath and sample temperatures were recorded. The temperature of the water bath was continuously regulated to give linearly increasing rates in the sample of 17, 39 or 93°C/hr. In all cases, when the sample reached 70°C, the heating was stopped. The beakers were removed and placed in a container with ice, which was placed in a cooler at 1°C overnight. Samples were run in triplicates, and each treatment had three replications.

Gel Strength

Gel strength was evaluated by the compression-extrusion method (Bourne, 1982) sometimes referred to as back extrusion (Acton et al., 1981) or annular pump (Harper et al., 1978; Steffe and Osorio, 1987). An Instron (Model 1122) Testing device was used and gel strength was measured by using a plunger 35 mm in diameter; compressing the protein gel twice, in a 45 mm diameter beaker, at a constant speed of 100 mm/min to a final 20 % of its original height. The highest peak of the first compression curve gave the force needed to compress the protein gel (to about 80 % of its height), and gel strength was recorded in N/cm^2.

Water Loss

After gel strength was measured, the gels were transferred to a 25x100 mm tube and centrifuged at a low speed of 476 x g for 10 min. Centrifugation accomplished the separation of water expelled from the surfaces of the gel by compression from the Instron. Low-speed centrifugation was used to minimize structural breakdown from centrifugal forces. The residue was weighed and the liquid loss expressed as the difference in weight from the initial 30 g sample.

Protein Loss

A 1 mL aliquot of the liquid expelled from the gels and separated by centrifugation was taken, and protein concentration was measured by the Biuret method. The expelled liquid also was examined, using SDS-PAGE, for characteristics of the proteins contained in the expelled liquid phase.

Statistical Analysis

The experiment was replicated three times and results were analyzed using a 2x3x4 factorial design with two protein conditions, three heating rates and four protein concentrations. The Statistical Analysis System (SAS, 1986) was used to determine means, standard errors and analysis of variance. Least significant difference ($P<0.05$) was used as a method of means separation.

RESULTS AND DISCUSSION

Muscle pH

The pH of PSE pigs was 5.6 at 45 min post mortem versus 6.2 for normal pigs. Ultimate pH for muscle samples was 5.5-5.6 and 5.5 to 5.7 for PSE and normal, respectively. The pH of protein extracts in this study was 6.1.

Protein Solubility

Protein solubility for salt-soluble extracts ranged from 48 to 54 mg/ml for normal pigs. Protein solubility for PSE pigs ranged from 34 to 54. The wider range of protein solubility for PSE pigs may reflect different degrees of stress that the PSE pigs experienced. Many researchers have suggested that loss of functionality from PSE is due to denaturation of myofibrillar proteins and to a loss of protein solubility (Penny, 1967; Penny, 1969; Park et al., 1975). To evaluate and compare proteins at equivalent concentrations, protein concentrations of 23 and 34 mg/ml from normal pigs and 23 mg/ml for PSE pigs were prepared as dilutions from more concentrated protein samples.

Gel Strength

Gel strength of heat-induced gels from halothane positive and halothane negative pigs are shown in Fig. 1 as a function of heating rate and protein concentration. There was a highly significant difference ($P<0.01$) due to protein concentration as expected. Gel strength increased in a log-log fashion with the increase of salt-soluble protein concentration (in the range 23 to 54 mg/ml) for both PSE and normal pigs; however, the average gel strength for protein extracts

from PSE pigs was about 45% of those from normal protein extracts at the same protein level. The force needed to compress the gels at 23 mg/ml protein was 0.24 N/cm^2 (avg.) for the PSE sample while 0.67 N/cm^2 (avg.) was required for the normal sample; at 54 mg/ml protein an average force of 2.9 N/cm^2 and of 6.3 N/cm^2 were needed for PSE and normal samples, respectively. This means that even when protein concentration was the same, the state of the protein was not, indicating that native protein from PSE muscle was considerably less functional even after solubilization. Consequently, the loss of functionality due to PSE is more than simply less solubility but involves some loss of molecular functionality. Stabursvik et al. (1984) found that part of the myosin molecule from PSE pork underwent extensive denaturation mainly in the light meromyosin segment and demonstrated a 50% reduction in apparent enthalpy. Park et al., (1975) also found that the rheological hardness properties of sausages made from PSE muscle were much less than those from normal muscles reflecting the biochemical properties of myofibrillar proteins (mainly the denaturation of the actomyosin system).

Heating rate had a significant effect (P<0.01) on gel strength of both PSE and normal protein extracts. Although the PSE protein was less functional, the effect of heating rate on gel strength still could be observed. In the protein range of 23 to 54 mg/ml, the 17°C/hr heating rate always resulted in the greatest gel strength, while the 93°C/hr heating rate resulted in the lowest gel strength. The intermediate heating rate of 39°C/hr always resulted in intermediate gel strength values. The force needed to compress the protein gel at 17°C/hr was 3.09 N/cm^2 for the PSE samples and 6.9 N/cm^2 for the normal samples; 3.07 N/cm^2 for the

PSE sample and 6.2 for the normal samples at 39°C/hr, and 2.6 and 5.78 N/cm^2 for PSE and normal samples respectively at 93°C/hr.

Gels were formed from PSE protein extracts at all protein concentrations in the present study. Although the fastest heating rate of 93°C/hr resulted in the weakest gel, there was no discontinuous phase or gel separation. There was enough protein at 23 mg/ml to achieve protein-protein interactions and gel formation, even though protein from PSE pigs was considerably less functional than from normal pigs.

Damage inflicted to the gel structure after compression was estimated by measuring the ratio of the second-compression value to the first-compression value. The highest peak of the second-compression curve also was measured in this study (Fig. 2). Gel strength from the second-compression cycle was directly proportional to protein concentration, as it was for the first compression cycle, of both PSE and normal gels. The force needed to compress the gel for the second time ranged from 23 to 40% of that needed for the first compression. There was no heating rate effect on the second-compression curve in this study.

Water Loss

There was an average of 15% more water loss from compressed gels for the PSE samples than for control samples as shown in Fig. 3. This indicates that water was not as effectively trapped by the extracted proteins in PSE samples as it was by the normal samples. This may mean that the proteins are less functional due to partial damage from PSE or that there is a selective effect of PSE on soluble proteins such that those proteins which remain soluble are less functional proteins. The SDS gels were utilized in this study to investigate these

effects. Penny (1969) found that water-holding capacity of myofibrils is largely determined by the extent of myofibrillar protein denaturation as a result of pH decline. Protein concentration had an effect on percent water loss from the PSE samples. Water loss from compressed gels increased with decreasing protein concentration. The average percent water loss from PSE samples was 60% at 23 mg/ml protein compared to 47% at 54 mg/ml protein. In the case of normal samples, average water loss was also higher at low protein concentrations (44% at 23 mg/ml protein), but remained relatively constant at 37% when protein concentrations were over 34 mg/ml protein. There was no heating rate effect on water loss in either case.

Protein Loss

Protein loss in the expelled liquid after compression and centrifugation as a function of protein concentration and heating rate for both PSE and normal samples is shown in Fig. 4. In all cases, at any protein concentration and at any heating rate, normal samples lost less protein in the expelled water than PSE samples (2.68 mg/ml and 3.63 mg/ml of protein in the water for 23 and 54 mg/ml protein in non-PSE samples at 17°C/hr), indicating that there was less protein-protein interaction occurring in the PSE samples. There was a significant difference (P<0.01) due to the effect of protein concentration. Protein loss was lower for both PSE and normal samples at low salt-soluble protein concentrations (3.06 mg/ml for 23 mg/ml protein in PSE samples at 17°C/hr) than at high protein content (4.11 mg/ml for 54 mg/ml protein in PSE samples at 17°C/hr) in the range studied.

Heating rate also had a significant effect on protein loss. Slow heating rates resulted in lower protein loss, for both PSE and normal samples, in the protein range and heating rates studied (3.74 and 3.22 mg/ml protein in the expelled water for 48 mg/ml protein in PSE and normal samples, respectively, at 17°C/hr), while faster heating rates gave higher protein losses (4.07 and 3.73 mg/ml protein in the expelled water for 48 mg/ml protein in PSE and normal samples, respectively, at 93°C/hr). The interactions of the proteins that result from applying a slow heating rate, in both PSE and normal samples, may be more complete and efficient than those from faster heating rates, allowing an extensive 3-dimensional gel structure to form, thus contributing to both increased gel strength and decreased protein loss in the expelled water.

SDS Electrophoresis

Figure 5 shows the SDS-PAGE patterns of a high molecular weight standard mixture (lane 1), and protein profiles from normal pigs (lanes 2-9). Lanes 2 and 3 correspond to extracts of salt-soluble proteins containing both myofibrillar and sarcoplasmic proteins, and lanes 4-9 to the fraction of soluble proteins in the expelled water of heat-induced gels after compression.

Lanes 2 and 3 show the different protein profile of salt-soluble-protein extracts (34 and 48 mg/ml, respectively) containing a mixture of sarcoplasmic and myofibrillar protein before heat treatment. Myosin was the major protein fraction in these extracts. There was no difference in this protein profile neither between samples from different pigs or between the various dilutions.

This protein profile changed drastically after heat treatment. Proteins that did not interact in making the three-dimensional-protein network during heat

treatment were expelled in the water after compression (lanes 4-9). It can be seen that all of the myosin (myosin heavy chains) was used to form the continuous protein network created by protein-protein interactions, conferring most of the strength to the gel network as has been reported elsewhere (Samejima et al., 1984; Asghar et al., 1985). Other myofibrillar proteins were the major protein fractions that remained soluble in water after heat treatment. Some actin remained soluble in the expelled water. Tropomyosin (β- and α-isoforms) also was found in the expelled water. This agrees with the findings of Samejima et al. (1982). They found that tropomyosin does not influence rigidity or gel strength of heat-set gels, and that tropomyosin is freed from the sediment (gel) after centrifugation of the heat-induced gel. This shows the high stability of this protein to heat denaturation (Yasui et al., 1982) and a lack of involvement with heat-set gels. Other myofibrillar proteins still soluble in the expelled water were myosin light chains (MLC1 and MLC3) and troponins I and C. Similar results were reported by Samejima et al. (1982) who found light chains remaining soluble after heat treatment. Samejima et al. (1984) supported Locker's proposition (Locker, 1956) that upon heating of myosin, myosin heavy chains aggregate and gel while light chains dissociate and solubilize.

The sarcoplasmic protein fraction can be seen, to a lesser extent than myofibrillar, in the expelled water after heat treatment. Porcine albumin is the major sarcoplasmic protein that still remains in the soluble state. There is another major sarcoplasmic protein that can be observed in Fig. 5 between MLC1 and troponin-I.

There was no significant difference between protein extracts from normal and PSE pigs before heating as shown in Fig. 6. Lane 1 is a high molecular

weight standard mixture. Lane 2 corresponds to a sample of an extract of salt-soluble proteins (54 mg/ml) containing both myofibrillar and sarcoplasmic proteins from normal pigs. Lanes 3 and 4 show the same protein pattern, but from PSE pigs, of samples from extracts of different protein concentrations (36 and 42 mg/ml, respectively) before heat treatment.

Lanes 5, 6, and 7 correspond to the soluble protein fractions, from heated PSE samples, that were expelled in the water after compression. They correspond to the different heating rates of 17°, 39°, and 93°C/hr, respectively. There was no visual difference of the protein profile in the expelled water between PSE and normal pigs as a result of the heating rate.

CONCLUSIONS

Textural and firmness characteristics of thermally induced gels, utilizing protein mixtures of sarcoplasmic and myofibrillar proteins from halothane positive (PSE) pigs were modified as a result of heating rates and different protein concentrations.

The PSE condition showed a highly significant effect ($P<0.01$) on gel strength, water retention, and soluble protein loss in the exuded water of thermally induced gels at protein concentrations equivalent to controls. Salt-soluble protein extracts from PSE muscle showed considerably less functionality since gel strength was lower (55%), while water loss and soluble protein loss in the exuded water were higher (13%, and 11%, respectively) than for gels made from protein extracts from normal pigs. Therefore, the protein, even after solubilization with salt, is in a less functional form. This was due to some partial protein denaturation as a result of PSE condition.

Heating rate was found to have a significant effect on gel strength, and on protein loss in the exuded water after compression of salt-soluble protein gels from PSE pigs. The greatest gel strength always resulted from using the slowest heating rate, while faster heating rates produced softer gels. Extracted proteins from PSE pigs responded, in the same manner as non-PSE proteins, to different heating rates although they are less functional. Foegeding et al. (1986) suggested that stronger 3-dimensional gels can be formed when applying slow heating rates because it may allow more protein-protein interactions to occur and result in a higher degree of order in the gel. Similar results were observed by Camou et al. (1989). This applies to protein systems extracted from PSE pigs as

well, although gel strength that results from PSE systems is of much lower quality than those from normal animals. Slow heating rates also allowed for minimum protein loss in the exuded water after gel compression, while increasing heating rates allowed for more protein loss.

There was a protein concentration effect on gel strength, water loss, and protein loss in the exuded water. Gel strength increased in a log-log fashion along with increasing salt-soluble protein concentration of the extract sample. Water loss decreased with increasing protein in the PSE samples, 60% at 23 mg/ml and 47% at 54 mg/ml. Soluble protein in the exuded water increased with increasing protein concentration, 3.21 mg/ml for a salt-soluble protein concentration of 23 mg/ml and 4.05 mg/ml in the expelled water for 54 mg/ml.

SDS electrophoresis did not show any significant difference in the protein profiles of either the extracted salt-soluble proteins or the exuded proteins from heated samples of PSE and normal pigs. This suggests that the differences found in this study, between gels from PSE and normal pigs, were caused by a general effect on proteins as a result of the PSE condition and not a protein-specific alteration affecting only a particular protein.

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Yasui, T., Ishioroshi, M., and Samejima, K. 1982. Effect of actomyosin on heat-induced gelation of myosin. Agric. Biol. Chem. 46(4):1049.

Figure 1.   Gel strength (first compression curve) of thermally induced gels from salt-

soluble protein extracts of muscles from normal and PSE pigs. [The

standard errors of the means for the measurements at protein

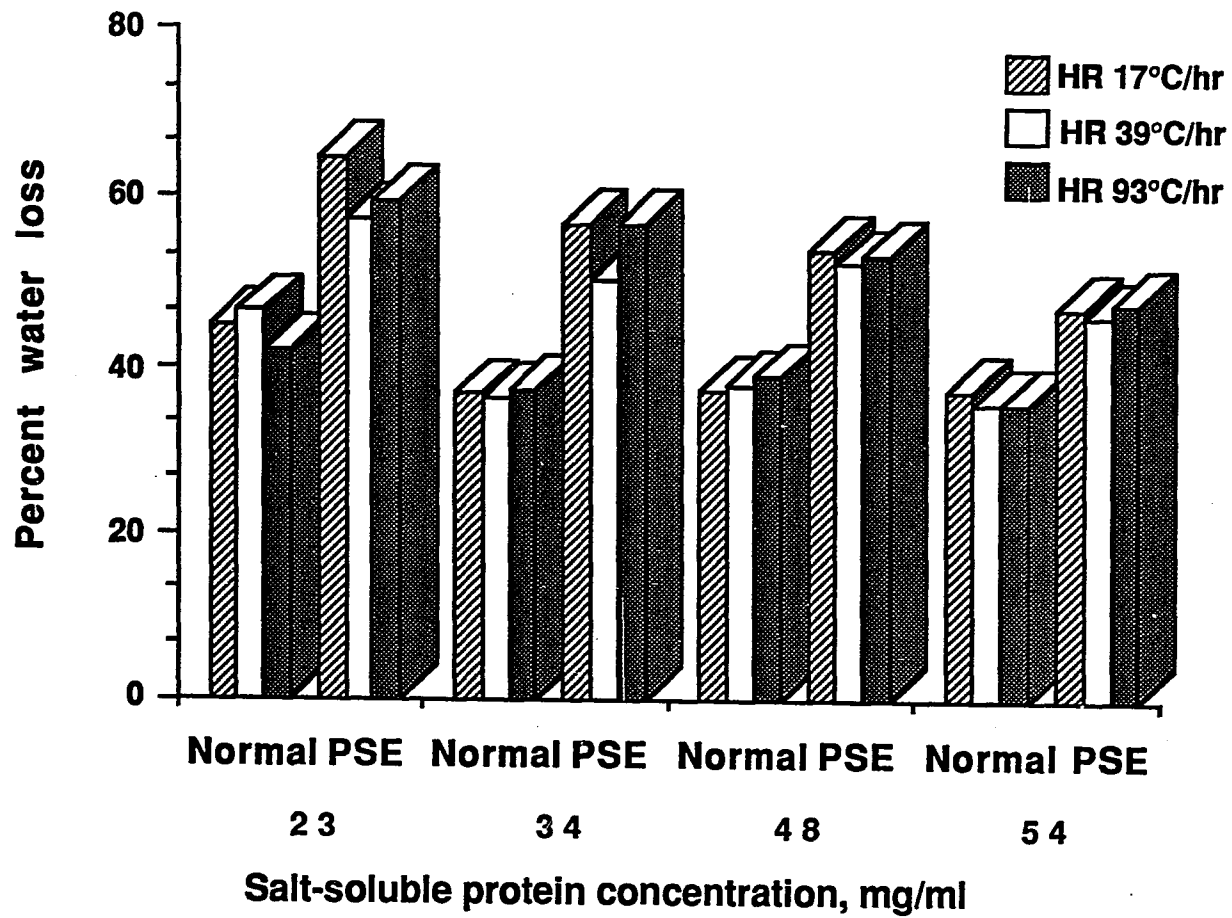concentrations of 23, 34, 48 and 54 mg/mL were 0.01, 0.03, 0.11,

and 0.09, respectively.]
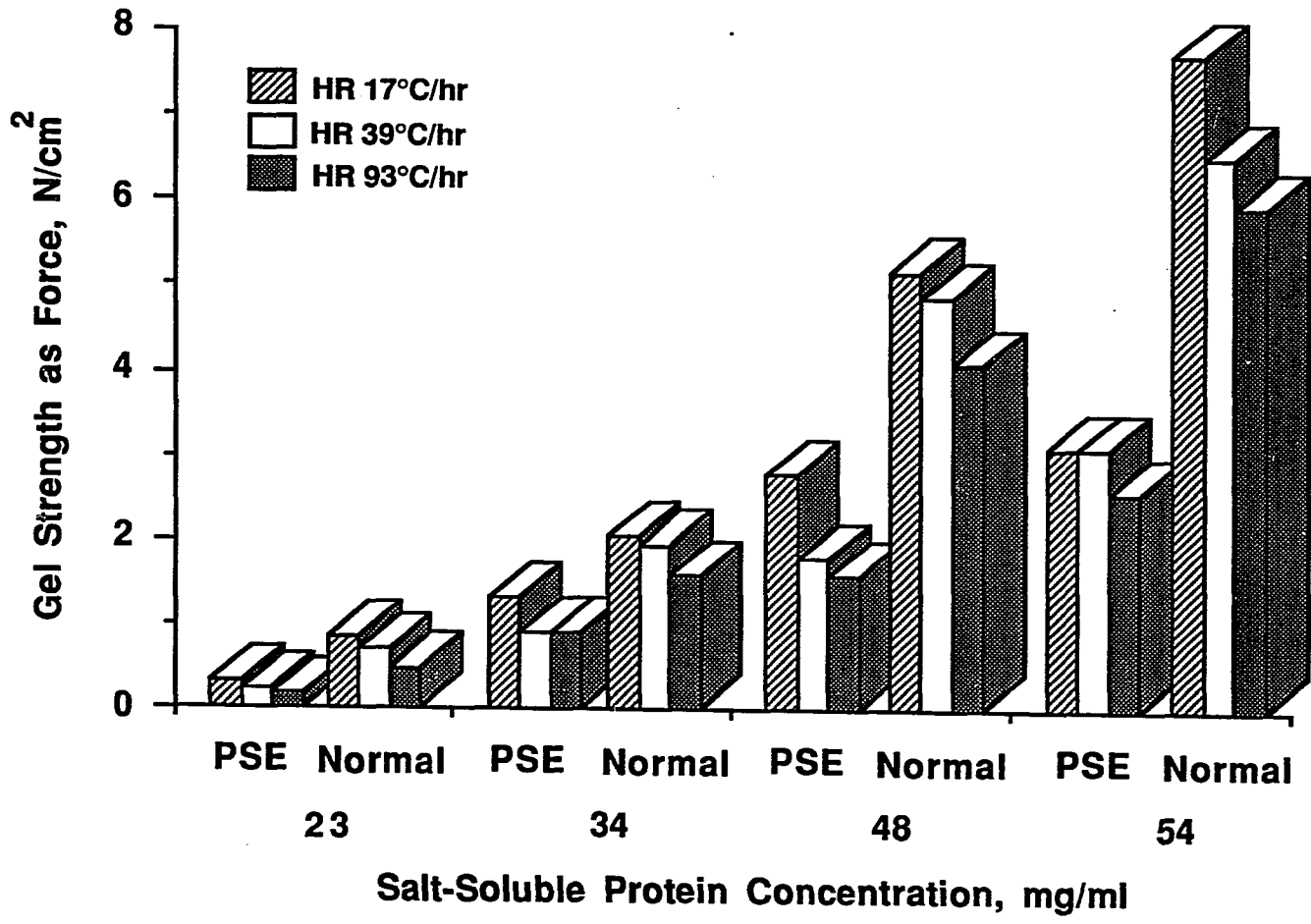
Figure 2.　Gel strength (second compression curve) of thermally induced gels from salt-soluble protein extracts of muscles from normal and PSE pigs. [The standard errors of the means for the measurements at protein concentrations of 23, 34, 48 and 54 mg/mL were 0.02, 0.02, 0.05, and 0.11, respectively.]
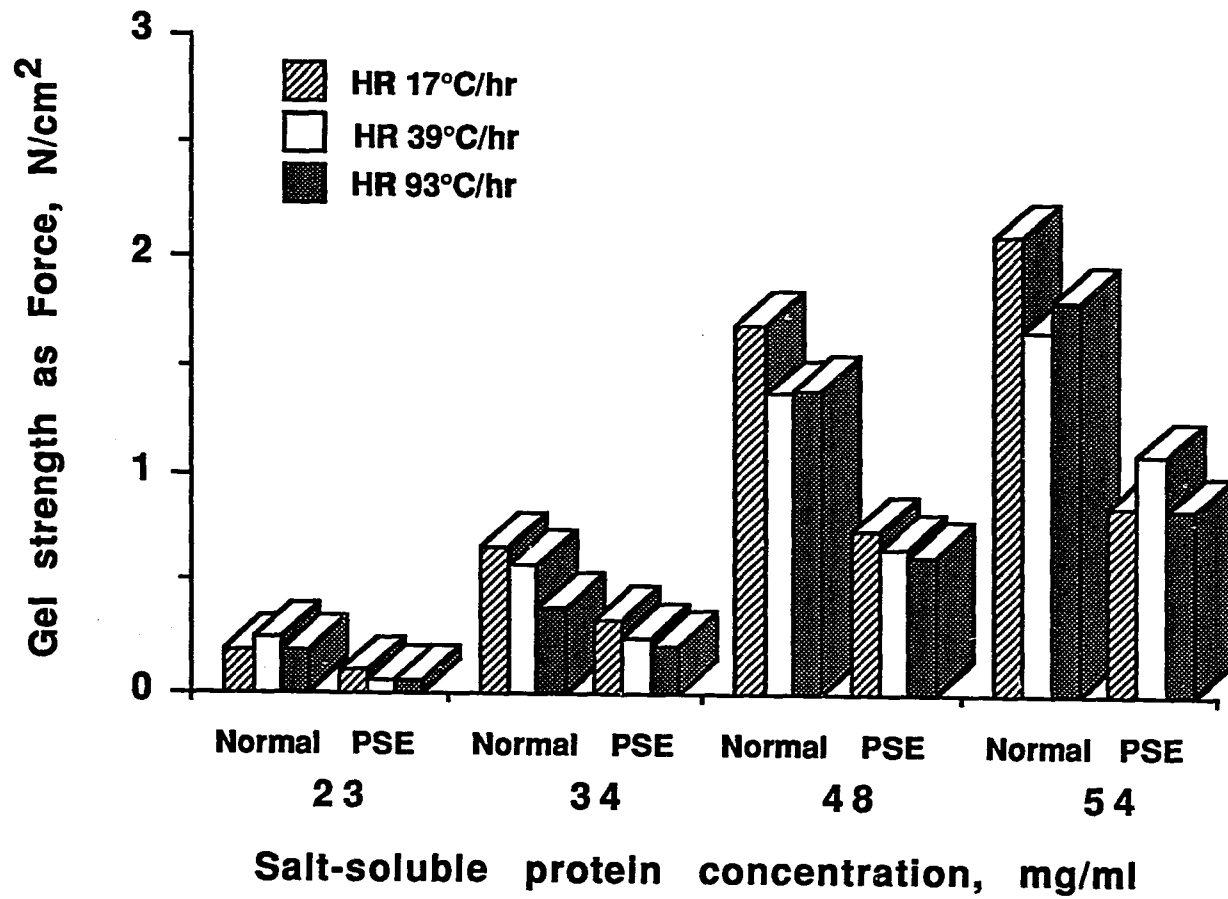
Figure 3.   Percent water loss of thermally induced gels from protein extracts of

muscles from normal and PSE pigs after gel compression. [The

standard errors of the means for the measurements at protein

concentrations of 23, 34, 48 and 54 mg/mL were 0.6, 0.72, 0.2,

and 0.35, respectively.]

Figure 4.   Protein content of expelled liquid of thermally induced gels from protein
            extracts of muscles from normal and PSE pigs after gel compression. [The
            standard errors of the means for the measurements at protein
            concentrations of 23, 34, 48 and 54 mg/mL were 0.01, 0.03, 0.11,
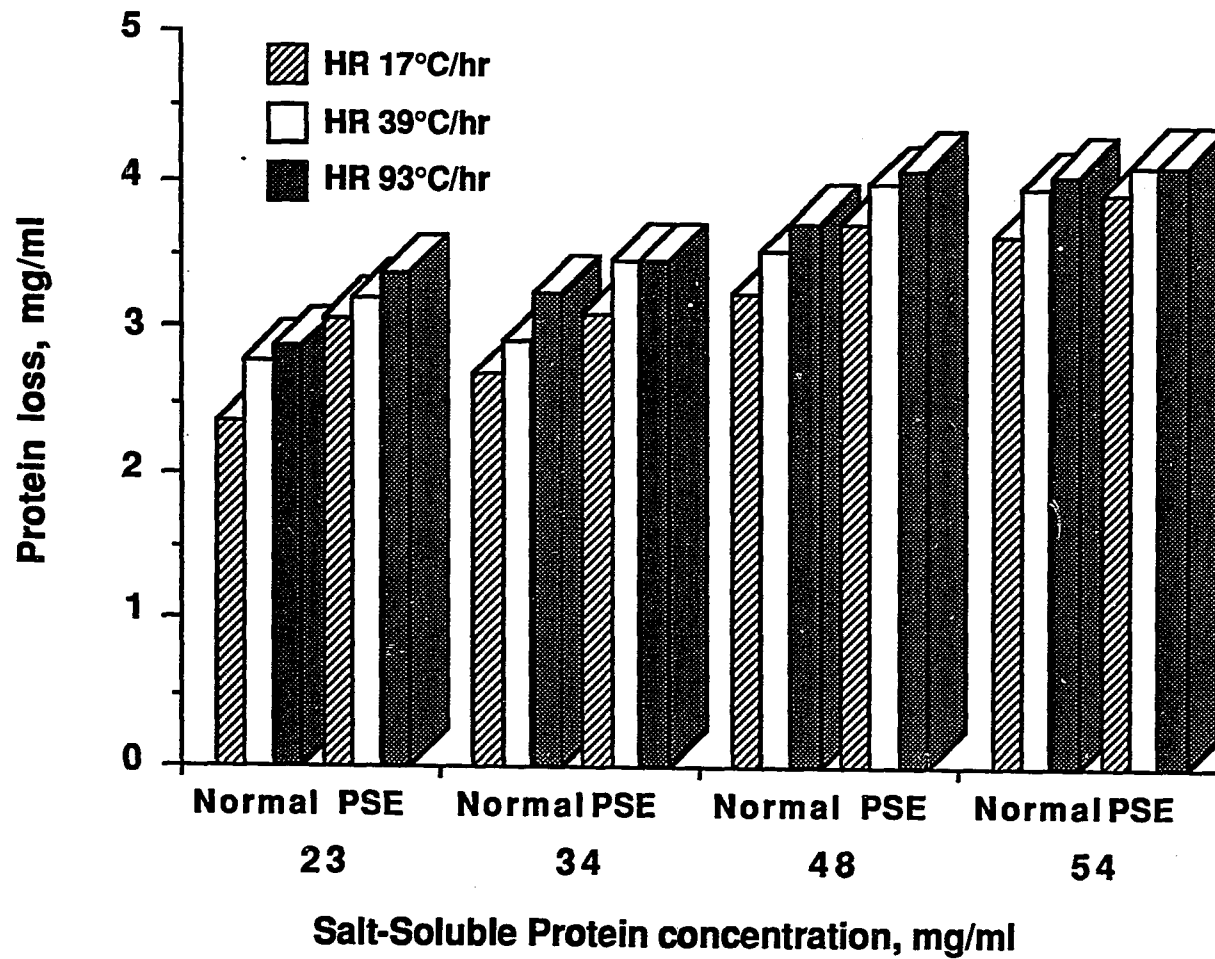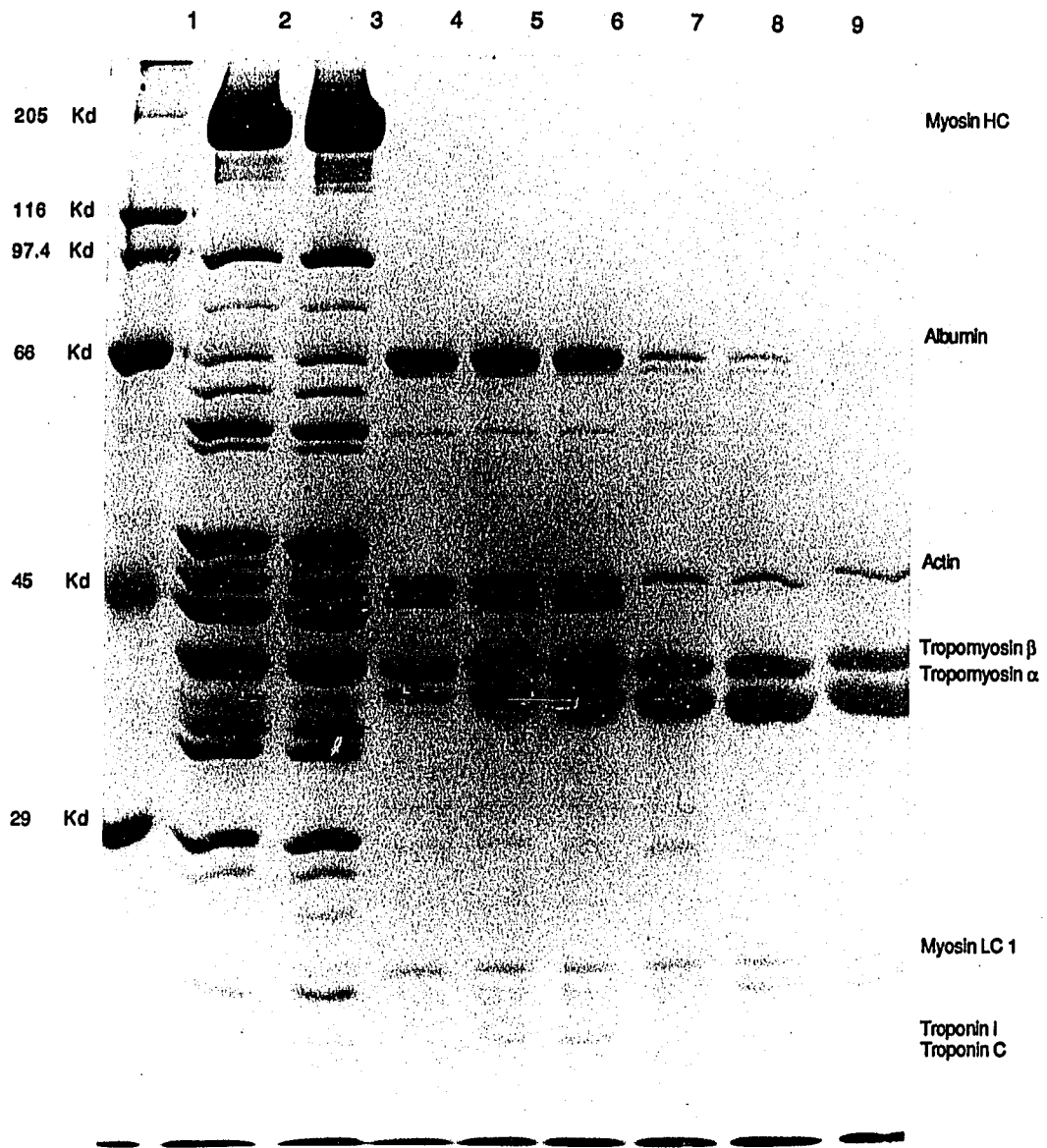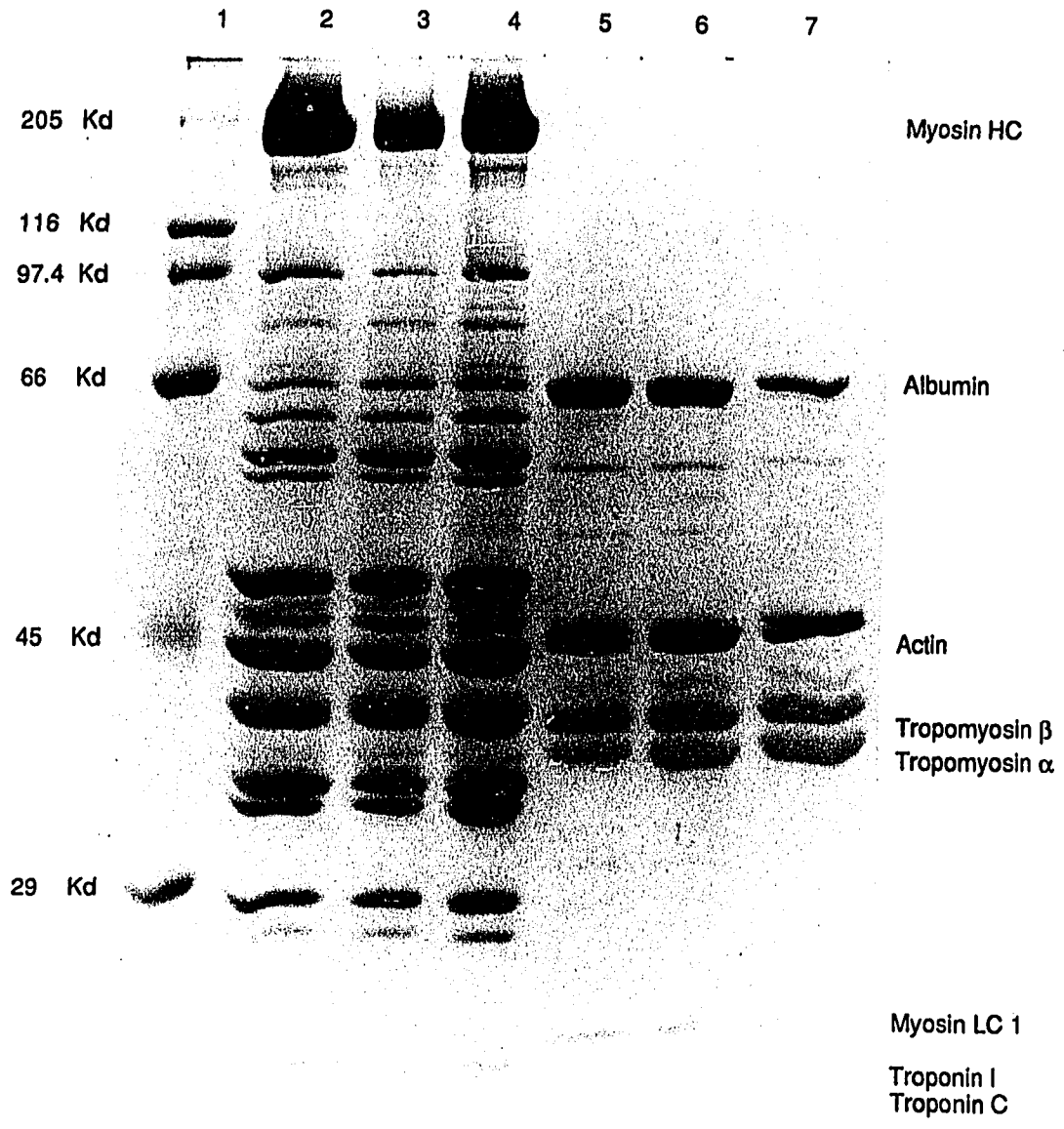   and 0.09, respectively.]

Salt-Soluble Protein concentration, mg/ml

Figure 5. SDS polyacrylamide gel of extracted-salt-soluble protein (sarcoplasmic

and myofibrillar protein) and soluble protein in the expelled water from

thermally induced gels from normal pigs after compression. [Forty μL of

protein solution were loaded in each well. Lane 1 contains standard

protein markers of 29 to 205 Kilodaltons. Lanes 2 and 3 correspond to

salt-soluble-protein extracts of 48 and 34 mg/ml. Lanes 4, 5 and 6 show

soluble protein in the released water when heating rates were 17, 39 and

93°C/hr respectively, from a protein solution of 48 mg/mL. Lanes 7, 8 and 9

show the respective heating rates from a protein solution of 34 mg/mL. The

amount of total protein loaded in each well was as follows lane (μg): 1(50),

2(72), 3(72), 4(128), 5(141), 6(149), 7(94), 8(111), 9(116).]

205 Kd

116 Kd
97.4 Kd

66 Kd

45 Kd

29 Kd

1   2   3   4   5   6   7   8   9

Myosin HC

Albumin

Actin

Tropomyosin β
Tropomyosin α

Myosin LC 1

Troponin I
Troponin C

Figure 6.   SDS polyacrylamide gel of extracted-salt-soluble protein (sarcoplasmic

and myofibrillar protein) and soluble protein in the expelled water from

thermally induced gels from PSE pigs after compression. [Forty μL of

protein solution were loaded in each well. Lane 1 contains standard

protein markers of 29 to 205 Kilodaltons. Lanes 2 correspond to a salt-

soluble-protein extract from normal pigs of 54 mg/ml. Lanes 3 and 4

correspond to the same protein profile (34 and 48 mg/mL, respectively) but

from PSE pigs. Lanes 5,6 and 7 show soluble protein in the released

water when heating rates were 17, 39 and 93°C/hr respectively, from a

PSE protein solution of 48 mg/mL. The amount of total protein loaded in

each well was as follows lane (μg): 1(40), 2(80), 3(72), 4(80), 5(134),

6(144), 7(146).]

1 2 3 4 5 6 7

205 Kd   Myosin HC

116 Kd

97.4 Kd

66 Kd   Albumin

45 Kd   Actin

Tropomyosin β
Tropomyosin α

29 Kd

Myosin LC 1

Troponin I
Troponin C

PART III. EFFECT OF NON-MEAT PROTEINS ON GELATION PROPERTIES OF PORCINE MUSCLE PROTEINS

EFFECT OF NON-MEAT PROTEINS ON GELATION PROPERTIES OF PORCINE MUSCLE PROTEINS

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Running title: Non-meat protein effect on gelation of porcine muscle proteins.

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ABSTRACT

The effect of soy protein isolate (ISP) and sodium caseinate (SC) on thermally induced protein gels made of extracted salt-soluble porcine muscle proteins were studied. Effects of heating rates (17, 39 and 93°C/hr) at various protein concentrations (32, 43 and 54 mg/ml) were evaluated for interactions between meat and non-meat proteins. Gel strength of protein extracts with 2% ISP added was increased about 24%; when 2% SC was added gel strength increased about 70%. Water holding capacity of protein extracts was improved by 37% and 4% when 2% SC and 2% ISP were added, respectively.

INTRODUCTION

Binders have been used in meat products mainly for yield improvement and to decrease raw material cost, among many other reasons (Everson, 1984; Sebranek, 1985; Amundson, 1988). However, special attention should be given to the effect that the binder in question will have with respect to the traditional product eating quality characteristics, like texture and flavor (Tuley, 1987). It is important that binders be used according to manufacturers instructions in order to maximize binder functionality and to result in a product with good quality characteristics.

The effects of different binders have been studied in a variety of meat products (Smith et al., 1973; Hermansson, 1975; Hermansson and Åkesson, 1975; Seideman et al., 1977; Sofos and Allen, 1977; Sofos et al., 1977; Comer, 1979; Siegel et al., 1979a; Siegel et al., 1979b; Comer and Dempster, 1981; Mittal and Usborne, 1985; Amundson, 1986; López de Ogara et al. 1986; Van den Hoven, 1987; Hoogenkamp, 1989), and in model systems with myosin or actomyosin complexes (King, 1977; Peng et al., 1982; Haga and Ohashi, 1984; Lin and Ito, 1985; Peng and Nielsen, 1986).

The work with meat products has evaluated many quality effects on non-meat proteins; however, gelation mechanisms responsible for many of these effects are difficult to study in intact meat mixtures. Most gelation studies have utilized simple purified protein solutions without consideration of the complex interaction of the mixture of muscle proteins. To study protein gelation of a muscle protein mixture, Camou et al. (1989) devised a model system utilizing a mixture of both sarcoplasmic and myofibrillar protein fractions. The same model

system was used for studying functional behavior of proteins from PSE pork (Camou and Sebranek, 1989) for gelation characteristics.

The objective of this study was to investigate the effect of isolated soy protein and sodium caseinate on quality characteristics, gel strength and water retention of thermally formed protein gels from pork. The extracted salt-soluble protein mixture, in this study, included both sarcoplasmic and myofibrillar fractions that closely resemble the extract fraction that occurs in processed meat products. The effect of different heating rates on extended samples also was evaluated to assess the potential for yield effects during heating of processed meats with extenders.

MATERIALS AND METHODS

Protein Extraction and Preparation

Semimembranosus muscles were separated from pork carcasses at the Meat Laboratory, Iowa State University, within 96 hr of slaughter. Samples were kept under refrigeration at 0-1°C until further use, not more than 12 hr.

Muscle samples were ground through a 4.5-mm plate, weighed and homogenized (Vita-mix 3600, Cleveland, OH) using 1 part of muscle with 2.2 parts of a 1°C solution containing 0.56M NaCl, 17.8mM $Na_5P_3O_{10}$, pH 8.3, and 1mM NaN_3 for 30 sec. This solution corresponds to 2.5% salt and 0.5% sodium tripolyphosphate, similar to levels used in commercial processed meat products. The mixture was placed in a cold room at 1°C for 1 hr to extract soluble protein. The mixture then was centrifuged (Beckman Model J-21C Centrifuge, Palo Alto, CA) at 12,000 x g, 2°C for 1 hr, and the protein extract separated from the residue and strained through gauze. This protein extract was kept overnight at 1°C until further use. The extract was examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for protein composition. Protein concentration of samples was adjusted by diluting the initial protein extract to the desired concentration range (32 to 54 mg/mL) with a salt-phosphate solution of the same ionic strength as the original.

The meat pH was determined by mixing 10 g ground muscle with 100 mL of deionized-distilled water for 5 min and measuring the slurry with a Fisher Accumet® selective ion analyzer (Model 750, E-5D pH electrode).

Samples of commercially available sodium caseinate and isolated soy proteins were added (in a dry form) at the 2% (w/v) level to each protein

concentration sample (liquid protein extract). This means that 19 mg of sodium caseinate was added per ml of extracted salt-soluble protein, while 18.6 mg of soy protein was added per ml. These binders were prepared 24 hours before being used to allow maximum hydration of these two proteins. After addition of these binders to the salt-soluble protein extracts, they were homogenized for 60 seconds at medium speed (set 5) using a Polytron homogenizer (Model PT 10/35, Brinkmann Instruments, Inc., Westbury, N.Y.). They were homogenized again, to solubilize the protein that precipitated, after 24 hours and just prior to the heat treatment.

Soluble Protein

The protein concentration of the extracted samples and their dilutions were measured by the Biuret method (Gornall et al., 1949). Protein concentration of the samples was recorded as mg of soluble protein per mL of solution.

SDS-Gel Electrophoresis

A discontinuous sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on protein extracts and proteins in the expelled water from heat-induced gels by using a modification of Laemmli (1970) with 4% acrylamide [bis-acrylamide/acrylamide, 1:30 (w/v)] stacking gel (pH 6.9) and 10% acrylamide [bis-acrylamide/acrylamide, 1:30 (w/v)] resolving gel (pH 8.9) of 14 x 12 x 0.15 cm dimension. Protein samples were incubated at 50°C for 20 min in 7.5% (v/v) 2-mercaptoethanol, 1.5% (w/v) SDS, 15% (w/v) sucrose, 0.3% (w/v) bromophenol blue, 15mM 2-N-morpholinoethanesulfonic acid (pH 6.5). The amount of protein loaded on the polyacrylamyde gel ranged from 35 to 164 µg in

a load volume of 35 µL. Electrophoresis was conducted at room temperature with 30 mA current per gel for 4.5 hr using Tris-glycine buffer (pH 8.6) as electrode buffer. Gels were stained in 0.2% Coomassie brilliant blue, 40% methyl alcohol, 7% acetic acid for 3-4 hr and destained in 40% methyl alcohol, 7% acetic acid for 5 hr. Molecular weights were estimated by comparing mobilities of proteins in the samples with mobilities of a high molecular weight standard mixture (Sigma, St. Louis, MO). This standard included a mixture of rabbit myosin (205K), ß-galactosidase (116K), phosphorylase B (97.4K), bovine albumin (66K), egg albumin (45K), and carbonic anhydrase (29K).

Heat Treatment

The heat treatment followed the procedures established by Camou et al. (1989). Aliquots (30 g) of the protein extract were placed in 100 mL beakers. Aluminum foil was placed on top of the beakers to avoid evaporation and water condensation during heating. The samples were allowed to equilibrate at room temperature for 30 min before heat treatment by submersion in a controlled temperature (20-25°C) water bath. Temperature was monitored by using an Omega digital thermometer equipped with multiple thermocouples. Both the water bath and sample temperatures were recorded. The temperature of the water bath was continuously regulated to give linearly increasing rates in the sample of 17, 39 or 93°C/hr. In all cases, when the sample reached 70°C, the heating was stopped. The beakers were removed and placed in a container with ice, which was placed in a cooler at 1°C overnight. Samples were run in triplicates, and each treatment had three replications.

Gel Strength

Gel strength was evaluated by the compression-extrusion method (Bourne, 1982) sometimes referred to as back extrusion (Acton et al., 1981) or annular pump (Harper et al., 1978; Steffe and Osorio, 1987). An Instron (Model 1122) Testing device was used and gel strength was measured by using a plunger 35 mm in diameter; compressing the protein gel twice, in a 45 mm diameter beaker, at a constant speed of 100 mm/min to a final 20 % of its original height. The highest peak of the first compression curve gave the force needed to compress the protein gel (to about 80 % of its height), and gel strength was recorded in N/cm^2.

Water Loss

After gel strength was measured, the gels were transferred to a 25x100 mm tube and centrifuged at a low speed of 476 x g for 10 min. Centrifugation accomplished the separation of water expelled from the surfaces of the gel by compression from the Instron. Low-speed centrifugation was used to minimize structural breakdown from centrifugal forces. The residue was weighed and the liquid loss expressed as percent water loss based on the weight of the gel.

Protein Loss

A 1 mL aliquot of the liquid expelled from the gels and separated by centrifugation was taken, and protein concentration was measured by the Biuret

method. The expelled liquid was also examined, using SDS-PAGE, for characteristics of the proteins contained in the expelled liquid phase.

Statistical Analysis

The experiment was replicated three times and results were analyzed using a 3x3x3 factorial design with three heating rates, three protein concentrations (salt-soluble protein extracts) and three protein mixtures (control, 2% ISP, and 2% SC). The Statistical Analysis System (SAS, 1986) was used to determine means, standard errors and analysis of variance. Least significant difference (P<0.05) was used as a method of means separation.

RESULTS AND DISCUSSION

Gel Strength

Gel strength of heat-induced gels with added non-meat proteins are shown In Figure 1 as a function of heating rate and protein concentration. Protein concentration had a highly significant effect (P<0.01) in all cases. Gel strength increased in a log-log fashion with the increase of salt-soluble protein concentration (in the range 32 to 54 mg/ml). The average gel strength for control gels was about 59% of those with 2% sodium caseinate (SC) and about 84% of those with 2% soy protein isolate (ISP). This was true when the extended samples were compared to the control in which they were solubilized. It should be noted that the extended samples contained more total protein. The control sample with 32 mg/ml salt-soluble protein was compared to an extended sample that contained 32 mg/ml salt-soluble protein plus 19 mg/ml of non-meat protein added resulting in total protein concentration of 51 mg/ml. In this case SC when added to protein extracts resulted in higher gel strength than ISP regardless of protein concentration or heating rate. The force needed to compress gels at 32 mg/ml protein was 2.2 N/cm^2 (avg.) for the controls while 3.5 N/cm^2 (avg.) and 3.1 N/cm^2 (avg.) were required when SC and ISP were added, respectively. At 54 mg/ml protein an average force of 5.9 N/cm^2 (avg.) was needed to compress the control samples while 9.9 and 6.6 N/cm^2 (avg.) were needed for compression of gel samples containing SC and ISP, respectively. Van den Hoven (1987) reported that caseinates increased muscle protein gel strength, and that caseinates did not interfere in gel formation. It should be noted that caseinates do not gel during heat treatment (Van den Hoven, 1987). SC is completely

soluble in water and its solubility is little affected by NaCl (Mittal and Usborne, 1985). However, at the ionic strength used in this study ($\mu=1.6$) ISP was not as well solubilized. Mittal and Usborne (1985) reported that ISP solubility is reduced significantly by the addition of NaCl. Utsumi and Kinsella (1985) found that increasing NaCl concentrations decreased the hardness of gels made of ISP partly due to the stabilizing effect that NaCl excerts on the thermally induced changes in structure of globulins in ISP.

Addition of non-meat protein binders to protein extracts has to be evaluated very carefully. In this experiment the addition of 2% SC meant that about 19 mg of SC per ml of extracted salt-soluble protein were added, and addition of 2% ISP equaled 18.6 mg/ml. The total protein content of the 32 mg/ml muscle protein extract with SC or with ISP added was about 51 mg/ml. Comparing gel strength of these two extended samples to the control of 54 mg/ml showed that gel strength was much lower for the former samples. Gel strength of the SC (32 + 19 mg/ml) sample was 59% of the control (54 mg/ml), while the ISP (32 + 18.6 mg/ml) was 52% of the control. This showed that replacing 2% of salt-soluble protein with 2% SC or 2% ISP resulted in a reduction of gel strength of 41 or 48%, respectively. This correlates with the findings of Comer (1979) where SC had positive effects upon cook stabilities of comminuted meat products, but a negative effect on texture. These binders should not be considered for replacing meat with good protein functionality but rather to supplement weak formulations.

Heating rate had a significant effect (P<0.01) on gel strength. In the protein range of 32 to 54 mg/ml, the 17°C/hr heating rate resulted in the greatest gel strength, while the 93°C/hr heating rate resulted in the lowest gel strength, and the intermediate heating rate of 39°C/hr resulted in intermediate gel strength

values. This was always true for control samples but for samples with added binders this was not always the case. This might depend upon how well the binders were solubilized in the salt-soluble protein extracts during the heat treatment. The force needed to compress the protein gel at 17°C/hr was 2.7 N/cm^2 for the control samples, 2.7 N/cm^2 for samples with ISP and 3.7 for samples with SC; 2.2 N/cm^2 for the control sample, 3.2 for sample with ISP and 3.6 for samples with SC at 39°C/hr, and 1.8, 3.4 and 3.3 N/cm^2 for control, with ISP and with SC samples respectively at 93 °C/hr. All these values represent samples with 32 mg/ml protein concentration.

The highest peak of the second-compression curve also was measured in this study (Fig. 2). Gel strength from the second-compression cycle was directly proportional to protein concentration, as it was for the first compression cycle. The force needed to compress the gel for the second time compared to that needed for the first compression ranged from 26 to 35% for controls, 46 to 59% for samples with ISP added, and 34 to 46% of samples with SC added . There was no significant heating rate effect on the second-compression curve in this study.

Water Loss

Percent water losses from compressed gels without and with binders are shown in Fig. 3. The addition of SC always resulted in the highest water retention when compared to controls or when ISP was added, at any protein concentration and at any heating rate. Soy isolate did not show any significant improvement on water retention at protein levels of 32 and 43 mg/ml, but it showed a small improvement (6% better than the control) at 54 mg/ml. This low

improvement in water retention might be due to a limited solubilization of ISP in the high ionic strength protein extract solution. López de Ogara (1986) found that the more soluble the soy protein in the system the lower the water loss from the heat induced gel.

Sodium caseinate improved water retention when compared to controls by 31, 42 and 38% (avg.) at 32, 43, and 54 mg/ml, respectively. It has been stated that SC proteins provide indirect water binding while providing direct fat binding (Hoogenkamp, 1984). However, in this model system where there is no addition of fat, SC might be interacting directly with protein in improving water binding. Water loss was much lower for the SC extended sample at 32 mg/ml (51 mg/ml total protein) than the control at 52 mg/ml. This showed that replacing 2% of salt-soluble protein with 2% SC may result in a reduction of water loss of 28%.

Protein Loss

Protein loss in the expelled liquid after compression and centrifugation as a function of protein concentration and heating rate for extended and non-extended samples is shown in Figure 4. In all cases, at any protein concentration and at any heating rate, control samples lost less protein in the expelled water than extended samples with ISP and SC (2.8, 4.4, and 7.2 mg/ml avg., respectively). The difference of this extra protein in the expelled water was due to ISP and SC remaining in that water, corroborated later by electrophoresis. Since SC was more soluble in the protein extract than ISP, samples with SC lost more protein than ISP, the latter being trapped as a solid in the gel network. These differences also meant that gels extended with ISP visually showed a granular appearance while gels extended with SC were very smooth. There was a significant

difference (P<0.01) due to the effect of protein concentration. Protein loss was lower for control and extended samples at low salt-soluble protein concentrations than at high protein content in the range studied.

Heating rate also had a significant effect on protein loss. Slow heating rates resulted in lower protein loss, for control and extended samples, in the protein range and heating rates studied. Faster heating rates gave higher protein losses. This is in accordance with the theory that interactions of the proteins that result from applying a slow heating rate, in both control and extended samples, may be more complete and efficient than those from faster heating rates (Camou et al., 1989), allowing an extensive 3-dimensional gel structure to form, thus contributing to both increased gel strength and decreased protein loss in the expelled water. These effects appear to hold true in all of the protein mixtures evaluated in this study.

SDS Electrophoresis

Figure 5 shows the SDS-PAGE patterns of a high molecular weight standard mixture (lane 1), soy isolate proteins (lane 2), and sodium caseinate (lane 3). Lane 4 corresponds to extracts of salt-soluble proteins containing both myofibrillar and sarcoplasmic proteins before heat treatment. Lanes 5-7 contain the fraction of soluble proteins found in the expelled water from controls of heat-induced gels after compression, while lanes 8-10 and lanes 11-13 correspond to the fraction of soluble proteins in the expelled water after compression of heat-induced gels from extended samples using ISP and SC, respectively.

Lanes 2 and 3 show the different protein profile present in the binders ISP and SC, respectively, before heat treatment. Isolated soy proteins contain mainly

two protein fractions, 11S (glycinin) and 7S (ß-conglycinin). Proteins that form the 7S (α-, α'-, and β-subunits) and the 11S (acidic and basic subunits) fractions can be seen in lane 2. Lane 3 shows β- and α-casein from the sodium caseinate used.

Lane 4 shows the protein profile from the salt-soluble protein extract (from control of 42 mg/ml) before heat treatment. This protein profile changed dramatically after heat treatment similar to earlier studies (Camou, 1989; Camou and Sebranek, 1989). In the non-extended samples (lanes 5-7) proteins from myofibrillar and sarcoplasmic fractions, that did not support formation of the three-dimensional protein network, were expelled in the water after compression. Myosin heavy chain was the major protein removed from the water and therefore involved in the protein network contributing most of the gel strength. Myosin heavy chain was not found in any case in the expelled water after heat treatment. The protein components identified in the expelled water were: actin, α- and β-tropomyosin , myosin light chains, troponin I and troponin C and may indicate limited contributions to gel formations.

Some of the sarcoplasmic proteins can be seen, to a lesser extent than myofibrillar proteins, in the expelled water after heat treatment. Most of this fraction formed a part of the protein network. Porcine albumin was the major sarcoplasmic protein that remained in the soluble state. Another common sarcoplasmic protein can be seen localized between myosin light chain 1 and troponin-I. Other sarcoplasmic proteins sometimes appeared in the expelled water but they were usually too faint to be identified and did not always appear in the electrophoresis gels.

Lanes 8-13 show protein fractions expelled in the water from extended gel samples after their compression. All of the soy protein subunits can be observed in these fractions (lanes 8-10) with the exception of the 11S basic subunit and the β-subunit from the 7S fraction. These subunits may be trapped in the gel forming part of the tridimensional network. Utsumi and Kinsella (1985) found that β-subunit of the 7S globulin had a high affinity for the basic subunit from the 11S globulin, and in this way they might interact and form part of the gel matrix. Haga and Ohashi (1984) found that upon heating first at 70°C and then at 100°C the denatured crude 11S protein associated continuously around the myosin B (actomyosin) network reinforcing it. Peng et al. (1982) reported that the basic subunits of the 11S interacted with myosin heavy chains while the acid subunits were not involved. However, this interaction occurred at temperatures higher than 85°C and when 11S protein was partially or fully dissociated. King (1977) reported the formation of a complex between both rabbit myosin heavy chain and some of the subunits of β-conglycinin (7S fraction) when they were heated at high temperature, 75-100°C. Peng and Nielsen (1986) found that interactions occurred between 7S and myosin at temperatures of 50-100°C. However, 7S interfered with the self aggregation of chicken myosin heavy chains (for a protein ratio 1:1, 7S:M) by forming a complex of 7S and myosin which remained in the supernatant rather than forming part of the three dimensional structural protein network. The latter point differs with the findings of the present study in which all myosin heavy chains formed part of the protein network and β-subunit from the 7S fraction was not found in the supernatant (expelled water). The protein ratio of salt-soluble protein to soy isolate in the present study ranged from 1.7:1 to 2.8:1 which was different from Peng and Nielsen (1986) study. These different

ratios may influence formation of the protein network. It has been suggested that the mechanisms involved in gelation of purified 7S fraction, purified 11S fraction and soy isolates (mixtures of 7S and 11S fractions) are different (Utsumi and Kinsella, 1985).

For SC extended samples (lanes 11-13) some β-casein was found in the expelled water. The α-casein subunit also remained in this water fraction but it seemed that this fraction did not interact as much in forming the protein network because of the high concentration still soluble after heat treatment. The expelled myofibrillar and sarcoplasmic fractions of the ISP- and SC-extended samples remained the same as the controls, indicating that the non-meat proteins did not modify the protein network formation.

CONCLUSIONS

The addition of 2% isolated soy proteins and 2% sodium caseinate to pork protein mixtures of sarcoplasmic and myofibrillar proteins was used to study the effect of these proteins on texture and firmness characteristics of thermally induced gels using different heating rates and different salt-soluble protein concentrations.

The addition of sodium caseinate had a dramatical effect on gel strength and water retention at any protein extract level. When compared to controls (for instance, 32 mg/ml), the addition of sodium caseinate (32 + 19 mg/ml) increased gel strength of the samples in the range 59-84%, and water retention was improved in the range 31-42%. These comparisons occurred when extended samples had higher total protein concentration than the control. However, it is not advisable to replace good quality meat proteins for the caseinate since gel strength might decrease if extended samples are compared with a control of same protein concentration, though water retention will still be high.

Isolated soy protein addition had an effect on gel strength but water retention was improved very little at higher concentration of extracted salt-soluble protein. Gel strength increased in the range 12-41% when compared to controls where non-meat proteins were solubilized, and water retention was improved in the range of 3-6%.

The difference on the effect of these two proteins may be due to the fact that sodium caseinate was totally solubilized in the high ionic strength model system, whereas soy isolate was just partially solubilized.

Heating rate was found to have a significant effect on gel strength on all of the controls as has been reported (Foegeding et al., 1986; Camou et al., 1989), and was found to have the same effect in some of the extended samples. This effect on extended samples was probably related to the solubility of the protein in the protein extract. There was a heating rate effect on protein loss in the exuded water after compression of control and extended samples. Slow heating rates allowed for minimum protein loss in the exuded water after gel compression, while increasing heating rates resulted in more protein loss.

A protein concentration effect was observed on gel strength, and on protein loss in the exuded water. Gel strength increased in a log-log fashion along with increasing salt-soluble protein concentration of the control and on the extended samples. Soluble protein in the exuded water increased with increasing protein concentration in all samples.

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Figure 1. Gel strength (first compression curve) of heat induced gels from extracted salt- soluble muscle proteins with addition of 2% sodium caseinate and 2% soy protein isolate. [The standard errors of the means for the measurements at protein concentrations of 32, 43 and 54 mg/mL were 0.2, 0.3, and 0.24, respectively.]
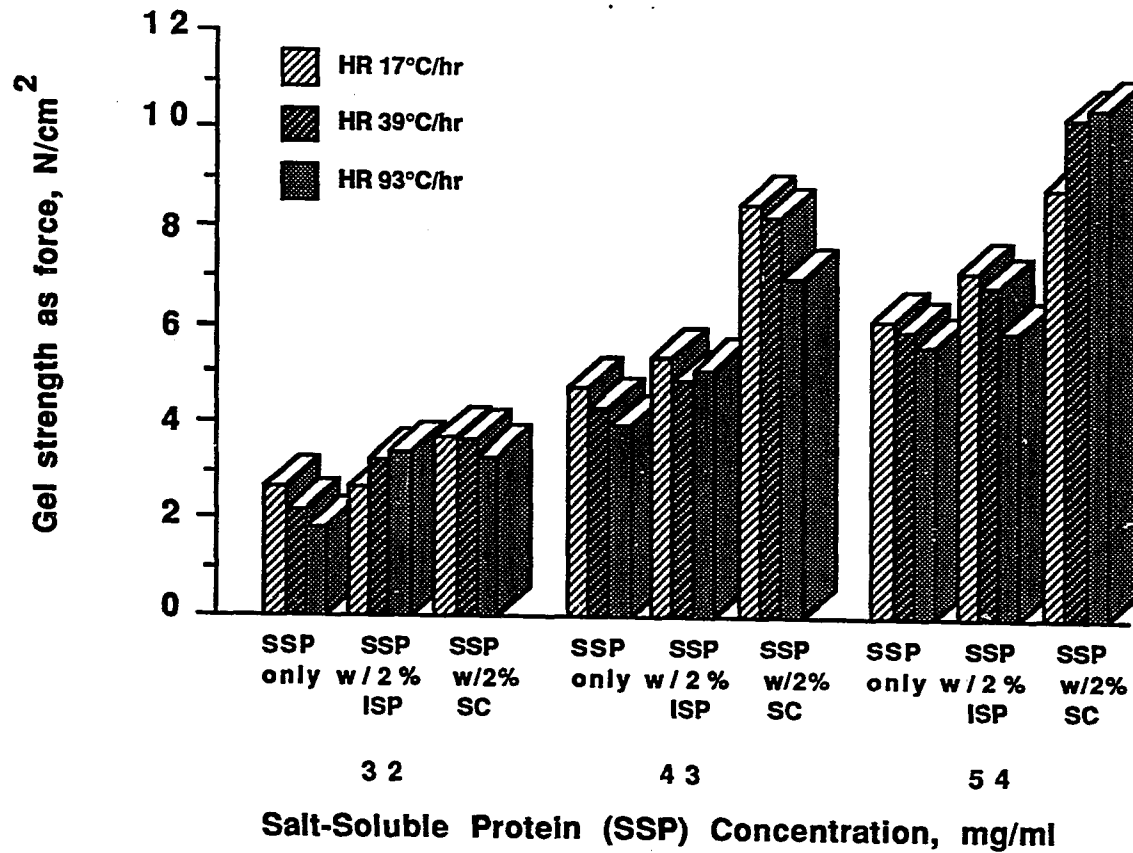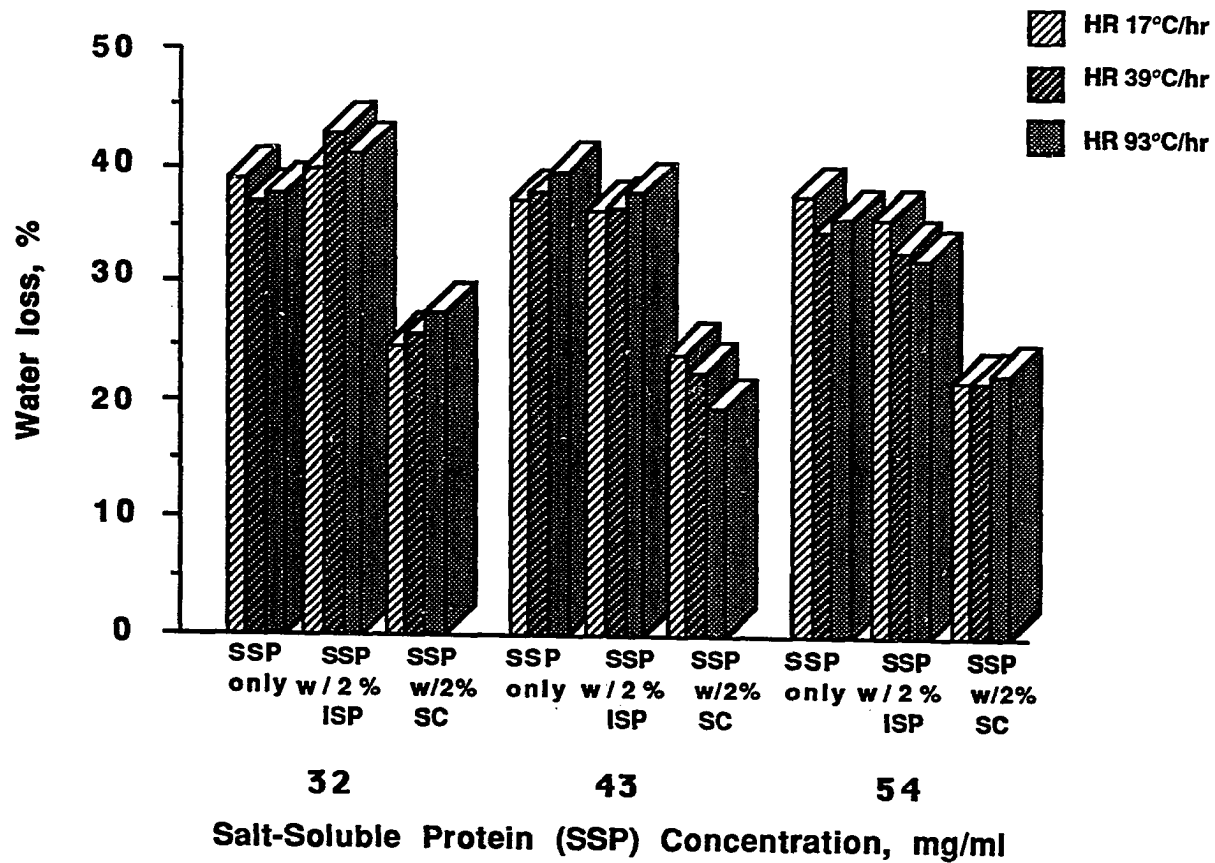
Figure 2.   Gel strength (second compression curve) of heat induced gels from extracted salt-soluble muscle proteins with addition of 2% sodium caseinate and 2% soy protein isolate. [The standard errors of the means for the measurements at protein concentrations of 32, 43 and 54 mg/mL were 0.1, 0.11, and 0.17, respectively.]
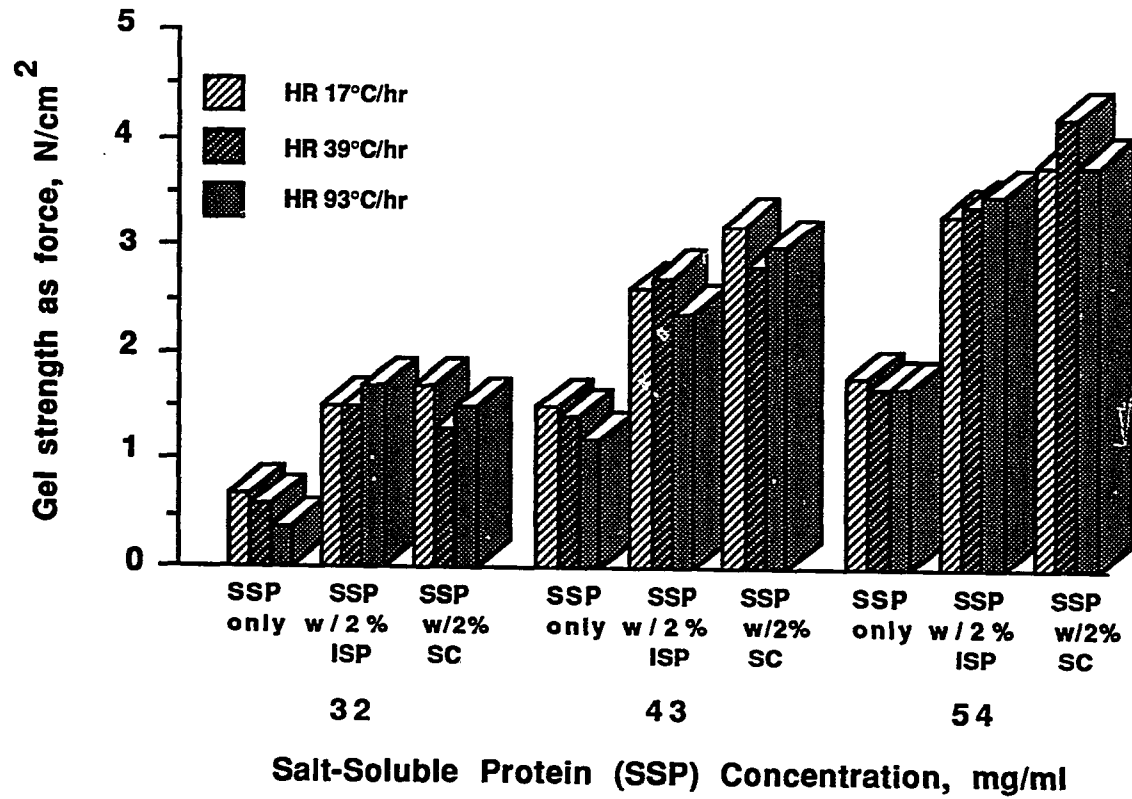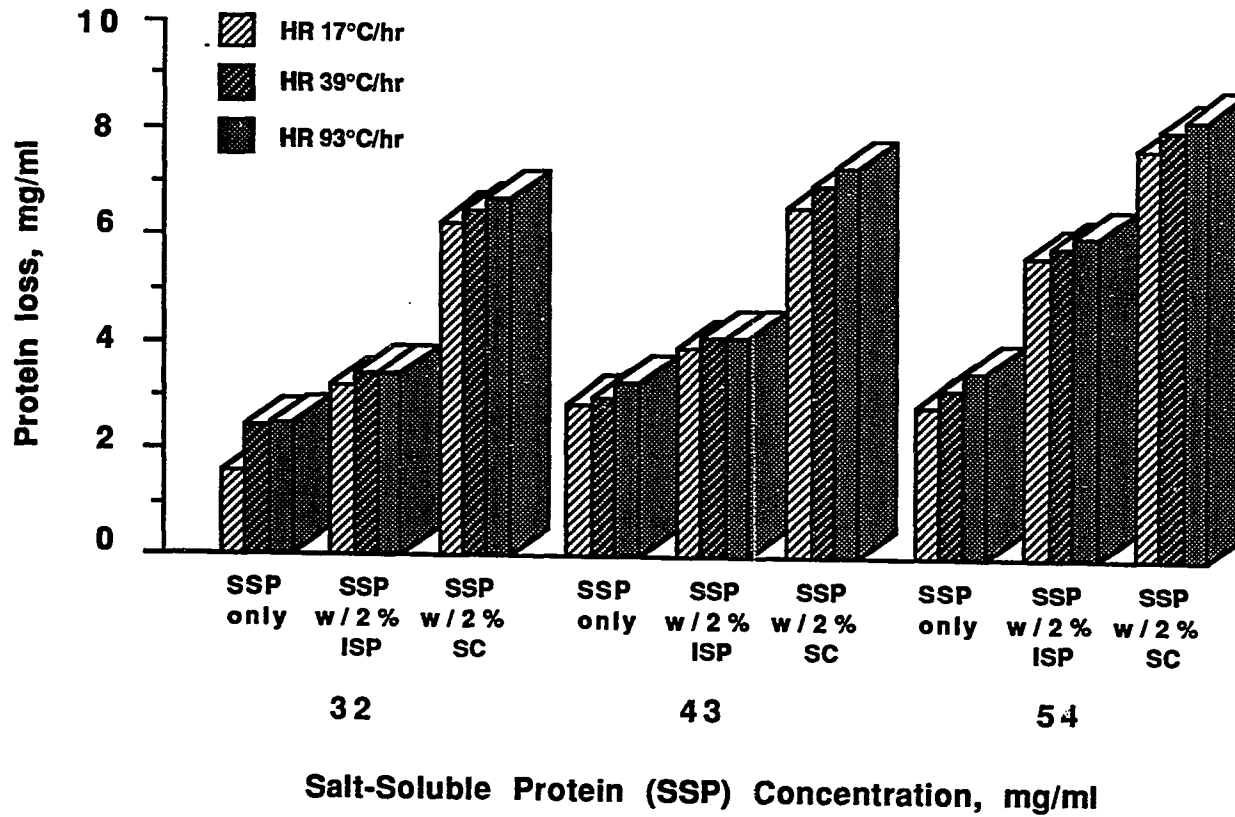
Figure 3.   Percent water loss of thermally induced gels from protein extracts of pork

muscle with addition of 2% sodium caseinate and 2% soy protein isolate

after compression. [The standard errors of the means for the

measurements at protein concentrations of 32, 43 and 54 mg/mL

were 0.7, 1.0, and 1.1, respectively.]

Figure 4. Protein content of expelled liquid of thermally induced gels from protein extracts of pork muscle with addition of 2% sodium caseinate and 2% soy protein isolate after gel compression. [The standard errors of the means for the measurements at protein concentrations of 32, 43 and 54 mg/mL were 0.9, 2.15, and 0.6, respectively.]

Figure showing a bar chart with "Protein loss, mg/ml" (y-axis, scale 0 to 10) versus "Salt-Soluble Protein (SSP) Concentration, mg/ml" (x-axis). Legend: HR 17°C/hr, HR 39°C/hr, HR 93°C/hr. X-axis categories: SSP only, SSP w/2% ISP, SSP w/2% SC, grouped under concentrations 32, 43, and 54.

Figure 5.   SDS polyacrylamide gel of extracted-salt-soluble protein (sarcoplasmic and

myofibrillar protein), non-meat proteins (2% sodium caseinate and 2% soy

protein isolate), and soluble protein in the expelled water from thermally

induced gels after compression. [Thirty to forty μL of protein solution were

loaded in each well. Lane 1 contains standard protein markers of 29 to 205

Kilodaltons. Lanes 2 and 3 contain the protein fractions of the soy protein

isolate and sodium caseinate used, respectively. Lane 4 correspond to the

salt-soluble protein extract of 42 mg/ml. Lanes 5, 6, and 7 show soluble

protein in the released water when heating rates were 17, 39 and 93°C/hr

respectively, from a protein solution of 42 mg/mL. Lanes 8, 9 and 10 show

the respective heating rates of the soluble protein in the released water of

samples extended with 2% soy protein isolate (from 42 mg/ml extract + 18.6

mg/ml ISP), and lanes 11, 12 and 13 show the same heating rates for soluble

protein in the released water of samples extended with 2% sodium caseinate

(from 42 mg/ml extract + 19 mg/ml SC). The amount of total protein loaded in

each well was as follows lane (μg): 1(55), 2(149), 3(133), 4(80), 5(137),

6(138), 7(140), 8(228), 9(232), 10(236), 11(263), 12(271), and 13(286).]

SUMMARY

Most of the research on the mechanisms of myofibrillar protein gelation have been conducted on pure myosin systems, or on systems containing myosin with different myofibrillar protein fractions. However, most of these studies have used low protein concentration (below 10 mg/ml) and constant heating temperatures. The objective of the current studies was to evaluate closely the effect of heating rates, protein concentration (>10 mg/ml), PSE condition, and addition of non-meat proteins on quality characteristics of thermally formed protein gels that closely resemble processed meat products. The extracted salt-soluble protein mixture in this study contained both sarcoplasmic and myofibrillar protein fractions that closely resemble the extract fraction that occurs in processed meat products. Water retention of gels was investigated to assess potential yield effects. Finally, SDS gel electrophoresis was utilized to confirm initial protein composition of salt-soluble protein extracts and to study the protein profile of soluble protein in the exuded water after compression of heat-set gels.

The first part of this study evaluated the effects of linearly increasing heating rates (17, 38 and 85°C/hr) and protein concentration (10, 20, 30, 40 and 50 mg/mL) on thermally induced gels made of extracted salt-soluble proteins. Gel strength analyses showed that the force needed to compress the heat-set muscle protein gels decreased when heating rate was increased. Water loss from compressed gels was higher at protein concentrations of 10 and 20 mg/mL than at 30 to 50 mg/mL. Water loss tended to be relatively constant after a protein concentration of 30 mg/mL was reached. Protein loss in the expelled water, after compression, was less for slow heating rates than for fast heating rates. The total

amount of protein in the expelled water increased with increasing protein concentration in the system. SDS gel electrophoresis demonstrated a drastic change in the protein profile after heat treatment. Proteins that did not interact in making the three-dimensional-protein network during heat treatment were expelled in the water after compression.

The second part of this study evaluated the effects of heating rates (17, 39 and 93°C/hr) at various protein concentrations (23, 34, 48 and 54 mg/ml) of thermally induced protein gels made of extracted salt-soluble proteins from stress susceptible pigs determined to be PSE. The average gel strength of heat-set gels made from PSE extracts was 45% of the controls at equivalent protein concentration. This showed that the state of PSE native protein was modified, and that PSE protein resulted in having much less functional properties when compared to those proteins extracted from normal hogs. Gel strength of normal and PSE-muscle protein gels from the first compression curve increased with increasing protein concentrations at all heating rates; however, gel strength was greater for slow heating rates than for fast heating rates in both PSE and normal samples. Percent water loss was greater for PSE extracts than for controls at the same protein concentration. Losses of 47% and 36% for PSE and controls, respectively, were observed at a protein level of 54 mg/ml protein. Water losses were not affected by heating rate in either case. Protein loss was lower, for both PSE and control, at low protein concentrations than at high protein content in the range studied. Slow heating rates decreased the amount of protein loss in the expelled water after compression, for both PSE and control, while faster heating rates increased the amount of protein loss in the exuded water. SDS gel electrophoresis did not show any significant difference in the protein profile

obtained between protein extracts from normal and PSE pigs before and after heat treatment.

The third part of this study evaluated the effect of soy protein isolate (ISP) and sodium caseinate (SC) on thermally induced protein gels made of extracted salt-soluble porcine muscle proteins. Effects of heating rates (17, 39 and 93°C/hr) at various protein concentrations (32, 43 and 54 mg/ml) were evaluated for interactions between meat and non-meat proteins. Gel strength of protein extracts with 2% ISP added was increased about 24%; when 2% SC was added gel strength increased about 70%. However, this was true when the extended samples (salt-soluble extract + ISP or SC) were compared to the control (salt-soluble protein) in which they were solubilized. It should be kept in mind that the extended samples, in this case, contained more protein than the controls. When extended samples were compared to controls at the same protein level then gel strength of the former samples were much lower than for the controls. Gel strength of extended sample with SC decreased 41% when compared to the control, while samples with ISP decreased 48%. Non-meat proteins should be considered for aiding weak or regular formulations, but not for replacing lean meat with good protein functionality. Water holding capacity of protein extracts was improved by 37% and 4% when 2% SC and 2% ISP were added, respectively. SDS gel electrophoresis showed that the 11S basic subunit and the β-subunit from the 7S fraction, both from isolated soy proteins, were not expelled in the water after compression of the thermally-induced gels indicating that these two subunits might be forming part of the three-dimensional protein network. SDS for SC extended samples showed that after the thermal treatment β-casein was the protein that interacted along with the salt-soluble proteins in the

making of the 3-dimensional protein network. Although, some of the β-casein still remained soluble in the expelled water. Most of the α-casein remained in the expelled water.

CONCLUSIONS

1. Gel strength increased with increasing amounts of extracted salt-soluble protein. This was true for protein extracts from normal and from PSE pigs in the protein range of 10 to 54 mg/ml.

2. The slowest heating rate (17°C/hr) resulted in increased gel strength at all protein concentrations. Gel strength decreased with increasing heating rates (39 and 93°C). This occurred in both protein systems: from normal and from PSE pigs.

3. Water loss after gel compression was constant for the systems with protein concentration above 30 mg/ml. However, this tended to increase when the system had a protein concentration below 30 mg/ml. No heating-rate effect on water loss was found.

4. Protein loss in the expelled water was higher at high protein concentration than at low protein concentration for all systems: including normal and PSE protein extracts, as well as for the extended samples with non-meat proteins.

5. SDS showed a dramatic effect between protein profiles before and after heat treatment. The protein profile after heat treatment included the proteins that did not interact in the formation of the 3-dimensional protein network. No significant difference was found in protein profiles from normal and from PSE pigs.

6. Salt-soluble proteins from PSE pigs had dramatically lower protein functionality than from normal pigs. Gel strength at equal protein concentrations from PSE pigs was about 55% less than that from normal pigs.

7. Water loss and protein loss in the exuded water were higher (13%, and 11%, respectively) for gels made from PSE protein extracts than from normal muscle extracts.

8. Addition of soy isolates and sodium caseinates increased gel strength and water retention of control samples. However, this was true when extended samples (salt-soluble extract + ISP or SC) were compared to controls (salt-soluble protein) in which they were solubilized. It should be kept in mind that the extended samples, in this case, contained more total protein than controls. When extended samples were compared to controls at the same protein level then gel strength of the former samples were much lower than for the controls.

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