This is to certify that the

dissertation entitled

Muscle Fiber Type and Ultimate pH of Two Bovine Muscles Influence Heat-Induced Gelation of Salt Soluble Proteins and Myosin

presented by

A. Virginia Vega-Vargas

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science

Denise M. Smith
Major professor

Date March 13, 1995

MUSCLE FIBER TYPE AND ULTIMATE pH OF TWO BOVINE MUSCLES INFLUENCE HEAT-INDUCED GELATION OF SALT SOLUBLE PROTEINS AND MYOSIN

By

A. Virginia Vega-Vargas

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

East Lansing, MI

1995

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ABSTRACT

MUSCLE FIBER TYPE AND ULTIMATE pH OF TWO BOVINE MUSCLES INFLUENCE HEAT-INDUCED GELATION OF SALT SOLUBLE PROTEINS AND MYOSIN

By

A. Virginia Vega-Vargas

The influence of muscle fiber type and ultimate pH on the thermal gelation properties of salt soluble proteins (SSP) and myosin from bovine vastus intermedius (VI, predominantly red muscle) and semimembranosus (SM, predominantly white muscle) at 0.6M NaCl and pH 6.05 (VI ultimate pH) or 5.50 (SM ultimate pH) were investigated. VI muscle had a higher fat content and lower protein content than SM. Solubility of VI and SM SSP decreased by 30% ($p<0.05$) between pH 5.8 and 5.6, and pH 5.6 and 5.4, respectively. Initial increases in storage modulus (G') of VI and SM SSP at pH 5.5 were at a lower temperature (about 36°C) than those at pH 6.0. The G' at 80°C varied with pH and muscle fiber type. VI SSP formed a more elastic gel network than SM SSP as indicated by tangent δ; however, tangent δ at pH 6.0 was lower than at pH 5.5 for both muscles. Stress and strain at fracture of SSP gels were the same ($p>0.05$) for SM at both pH's and VI at pH 5.5, but higher in VI at pH 6.0. SSP gels from both muscles had lower syneresis and expressible moisture at pH 6.0, VI had poorer water holding ability compared with SM at pH 6.0.

VI and SM myosin heavy and light chain contained different isoforms. Endotherms of VI myosin at pH 6.05 had three peaks with

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transition temperatures (Tm) of 53, 57, and 65°C, whereas at pH 5.50 two Tm of 42 and 58°C were observed. SM myosin at pH 6.05 and 5.50 had Tm at 46°/58°C and 43°C/50/62°C, respectively. Both myosin endotherms were deconvoluted in 10 two-state transitions. The aggregation rate was higher for SM than VI myosin. Aggregation started at a lower temperature at pH 5.50 than 6.05. The onset temperature for gelation (defined G'= 10 Pa) occurred at 38°C for SM myosin at pH 5.50 compared with 55°C for VI myosin at pH 6.05; however, G' at 80°C were similar. Both ultimate pH and fiber type influenced denaturation, aggregation, and gelation of bovine myosin during heating. Fiber type had a greater influence on the aggregation step than pH.

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Gracias Mamá,

DEDICATION

To my beloved mother who has always supported me in all aspects of this long adventure which culminated in my Ph.D. degree. Thank you, Mother. (A mi querida Mamá que siempre me ha apoyado en todos los aspectos durante mi larga aventura que culminó con mi doctorado. Gracias Mamá)

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ACKNOWLEDGMENTS

The author wishes to give special thanks to her major professor, Dr. Denise M. Smith, for her support during the Ph.D. program and her patience during the painful writing process.

Also, the author appreciates all the help from the members of the guidance committee: Dr. Gale M. Strasburg, Dr. Robert A. Merkel, Dr. Alden M. Booren and Dr. Mark R. Worden.

Sincere appreciation is extended to different faculty members who permitted the author to use their facilities: Dr. James F. Steffe, Dr. William G. Helferich, Dr. John E. Wilson, and Dr. Maurice R. Bennink.

Special thanks are extended to Mr. Thomas Forton who helped to obtain the raw materials for this research. Recognition is in order for different co-workers who teach me different techniques needed for this research: Dr. Chen-Hsing Wang, Dr. Shue-Fung Wang, Chin-Gin Low, Danilo Campos, Ross Santell, Hsiu-Ching Yang, and Li-Ju Wang.

Last but not least, the author wants to express her gratitude to Dr. Roscoe L. Warner for his unconditional support and help during the last two years.

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CHAPTER 1

INTRODUCTION

Skeletal muscle contains different fiber types: α-red (slow, type I), α-white (fast, type IIb), and ß-red (intermediate, type IIa). The proximate composition, pH and functional properties differ in muscles composed of different fiber types (Lyon et al., 1984; Maesso et al., 1970; Meyer and Egelandsdal, 1992; Ramsbottom and Strandine, 1948). The effect of muscle type on heat-induced gelation has been the subject of recent studies using simple systems, such as myosin or myosin subfragments, to more complex systems, such as whole muscles (Egelandsdal et al., 1994; Meyer and Egelandsdal, 1992).

Different muscle protein isoforms are present in each muscle fiber type (Pette and Staron, 1990). Myosin, which is shown to be among myofibrillar proteins to present the better functional properties such as gelation, exists as different isoforms that have different thermal behaviors. Rheological and water holding properties of muscle have been shown to be influenced by ultimate pH of the meat (Daum-Thunberg et al., 1992). Myofibrillar protein isoforms and ultimate pH have been suggested as possible reasons for variations in thermal gelation behavior of muscle proteins.

Proteins from white muscles form stronger gels than those from red muscles in several species. Differences in gel properties and myofibrillar protein composition of turkey and chicken red and white

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muscle have been reported. Most studies have been done using poultry breast and leg or thigh muscles as these contain predominately white and red fibers, respectively (Asghar et al., 1985; Foegeding, 1987; Xiong and Brekke, 1990a,b). It is known that there are different proportions of fiber types in bovine muscles. Consequently, there will be differences in gelling properties of bovine muscle (Egelandsdal et al., 1994; Fretheim et al., 1986; Meyer and Egelandsdal, 1992).

Most processed meat products are formulated using least cost formulation calculations which are primarily based on composition and cost. In this type of formulation, the processor tries to meet protein content specifications using any cut of meat available without considering the functional properties of the muscle. Cassens and Cooper (1971) suggested that a certain fiber composition might be optimal in the manufacture of processed meat products. Fiber type is known to affect the final texture of processed meats and their handling during processing (Xiong and Blanchard, 1994a).

A formulation based only on protein content does not account for specific functional properties of each muscle in the formulation. A greater percentage of one fiber with respect to the another may change final product texture characteristics. Similarly, pH has a great effect on gelation due to the three dimensional matrix resulting from electrostatic forces between protein molecules. So, a processed meat formulation based on muscle type and protein concentration may be more realistic in ensuring consistent yields and final product texture.

The influence of fiber type on heat-induced gelation in bovine muscle needs to be studied more extensively as the influence of muscle pH and protein isoform is not known. Most studies have been done in

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masseter (red fiber type) and cutaneus trunci (white muscle). We selected vastus intermedius (VI) and semimembranosus (SM) since these two muscles are more readily utilized in a processed meat product. porcine VI is composed of approximately 76% fiber type I (Robe and Xiong, 1992; Suzuki and Cassens, 1980) and bovine SM approximately 70% fiber type II (Iwamoto et al., 1991)

The overall goal of this project was to determine the effect of fiber type and ultimate muscle pH on the gelation properties of muscle proteins using bovine VI and SM muscle.

In the first study the objectives were to:
a) compare the composition and pH of bovine semimembranosus and vastus intermedius, b) determine the influence of pH on SSP solubility profile, and c) evaluate the effect of muscle type and ultimate pH on thermal gelation behavior of bovine muscle SSP.

The objectives of the second study were to:
a) identify myosin isoforms of bovine semimembranosus and vastus intermedius muscles, b) determine the influence of myosin isoform and pH during the three steps of heat-induced gelation; denaturation, aggregation and matrix formation or cross-linking.

The dissertation was organized around these two studies. The first three sections include the Abstract, Introduction and Literature Review for the entire dissertation. Each study was then organized as a manuscript with its specific abstract, introduction, materials and methods, results and discussion and conclusions section. The last common sections were Conclusions, Future Research and List of References in Journal of Food Science format for the entire dissertation.

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CHAPTER 2

LITERATURE REVIEW

2.1 MUSCLE COMPOSITION

Muscle is the principal component of meat. As a living tissue, muscle is composed of water, protein, fat, carbohydrate and inorganic constituents. In a typical mammalian muscle, water content is about 75% and protein content is about 19%. Carbohydrates, mainly lactic acid and glycogen, comprise about 1.2%; lipids about 2.5% and other soluble nonprotein substances about 2.3% (Lawrie, 1991).

Water is present within the three dimensional network of muscle fibers and is associated with connective tissue (Wismer-Pedersen, 1987). In beef muscles, as in other species, water and lipid content are inversely related; one increases at the expense of the other. Muscle lipids are present as triacylglycerides and phospholipids. Soluble nonprotein substances can be subdivided in nitrogenous nonprotein soluble substances (NPN) such as creatine and inorganic substances (minerals).

The composition differs between muscle types; red muscle types have been reported to have higher in lipid and moisture content and lower in protein content than the white muscle type among species (Cassens and Cooper, 1971; Meyer and Egelandsdal, 1992; Ramsbottom and Strandine, 1948). Moreover, the ultimate pH (pH after rigor onset) between muscle types has been shown to be different. The pH is 0.2 to

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0.5 pH units higher for red muscle types than white muscle types among species, including chicken and cattle (Amato et al., 1989; Foegeding, 1987; Lawrie et al., 1963; Meyer and Egelandsdal, 1992).

2.1.1 Muscle proteins

Muscle proteins are classified into three groups: myofibrillar, sarcoplasmic and stromal. In skeletal muscle, myofibrillar proteins constitute between 50-55% of the total protein, sarcoplasmic proteins about 30 to 34%, and the remaining portion is stromal or connective tissue proteins (Acton et al., 1983).

Sarcoplasmic proteins are in solution in the intracellular fluid and can be extracted with water or low ionic strength (0.1 μ or less) solutions at pH 6.7-7.5 (Pearson and Young, 1989). This fraction contains more than 100 different proteins, including myoglobin and metabolic enzymes (Scopes, 1970). These proteins can be classified into four different fractions: nuclear, mitochondrial, microsomal and cytoplasmic. In general, sarcoplasmic proteins are globular or rod shaped proteins and have low viscosities, low water-binding capacities, molecular weights between 20,000 and 100,000 and isoelectric points in the range pH 6.0 - 7.0 (Morrissey et al., 1987).

Myofibrillar proteins are an integral part of the muscle filaments and require a higher ionic strength (0.3 μ up to 0.5 μ) for extraction (Pearson and Young, 1989). They can be divided into three subgroups: contractile proteins, regulatory proteins and cytoskeletal or scaffold proteins. The contractile proteins, myosin and actin, account for about 50% and 20%, respectively (Obinata et al., 1981; Yates and Greaser, 1983) of the total myofibrillar protein. Myosin is responsible for most

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of the functional properties of processed meat (Smith, 1988). Actin influences myosin gelation even though it does not form a gel (Nuckles et al., 1991; Wang and Smith, 1994a). The regulatory proteins, including troponin, tropomyosin and the actinins, play a role in the initiation and control of contraction. Connectin, C-protein, myomesin, desmin, nebulin, and filamin are among the cytoskeletal proteins which provide the structure and alignment of the myofibrils.

Stromal proteins are insoluble in water and dilute salt solutions and include connective tissue and associated fibrous proteins. The major proteins in this group are collagen, elastin and keratin.

2.1.2 Myosin

Myosin is a highly asymmetric molecule with dimensions of about 150 nm length and 1.5-2.0 nm diameter in the rod portion and 8 nm diameter in the globular heads (Pearson and Young, 1989). The myosin molecule consists of six separate polypeptides, with a total molecular weight of about 521,000 daltons for rabbit skeletal myosin (Yates and Greaser, 1983). The two large polypeptides are called myosin heavy chains and four smaller ones are known as light chains. Myosin has an isoelectric point of 5.3 and contains a high percentage of glutamic and aspartic acid.

Each myosin heavy chain contains about 1934 amino acids resulting in a molecular weight of about 220,000 daltons (King and Macfarlane, 1987; Knight and Trinick, 1987) and contains an α-helix region known as the myosin rod or tail. Rod regions of the two heavy chains are intertwined in a right-handed twist to form a coiled-coil superhelix stabilized by knob-into-hole packing and hydrophobic interactions

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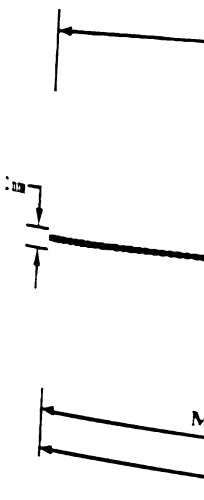
(Squire, 1986). The rod portion contains two different levels of repeating units. Seven repeating residues (a-b-c-d-e-f-g) form the backbone of the rod. Residues a and d are hydrophobic and are in the core of the molecule. The second level of repeat unit is composed of 28 residues which give origin to positive and negative charged stripes in the myosin molecule which are important in the formation of thick filaments (Squire, 1986). The function of the rod is to assemble myosins into thick filaments. A schematic diagram of myosin is shown in Fig. 2.1.

On the NH_2-terminal end, each heavy chain has a globular pear shaped region known as the myosin head which contains the ATPase active site and the actin-binding region. The three dimensional structure of the head of chicken pectoralis myosin has been determined using crystal x-ray diffraction and 48% of the residues were involved in α-helix conformation (Rayment et al., 1993). These α-helices are extended through the major part of the myosin head and act as light chain binding sites. Seven ß-strand motifs are also present and are located in the thick part of the myosin head. The two myosin heads are not necessarily identical (Inoue et al., 1977 and 1979). Up to four post-translational methylated residues are present in the myosin head: two trimethyllysine, one monomethyllysine and methylhistine (Knight and Trinick, 1987). The latter is only found in the type II myosin. These are not present in the rod portion (Hayashida et al., 1991). The function of these residues are unknown, but they are used to follow myofibrillar protein degradation (Wohlt et al., 1982).

Each myosin head contains two light chains, each with a molecular weight of about 20,000 daltons, which are located in the rod end of the



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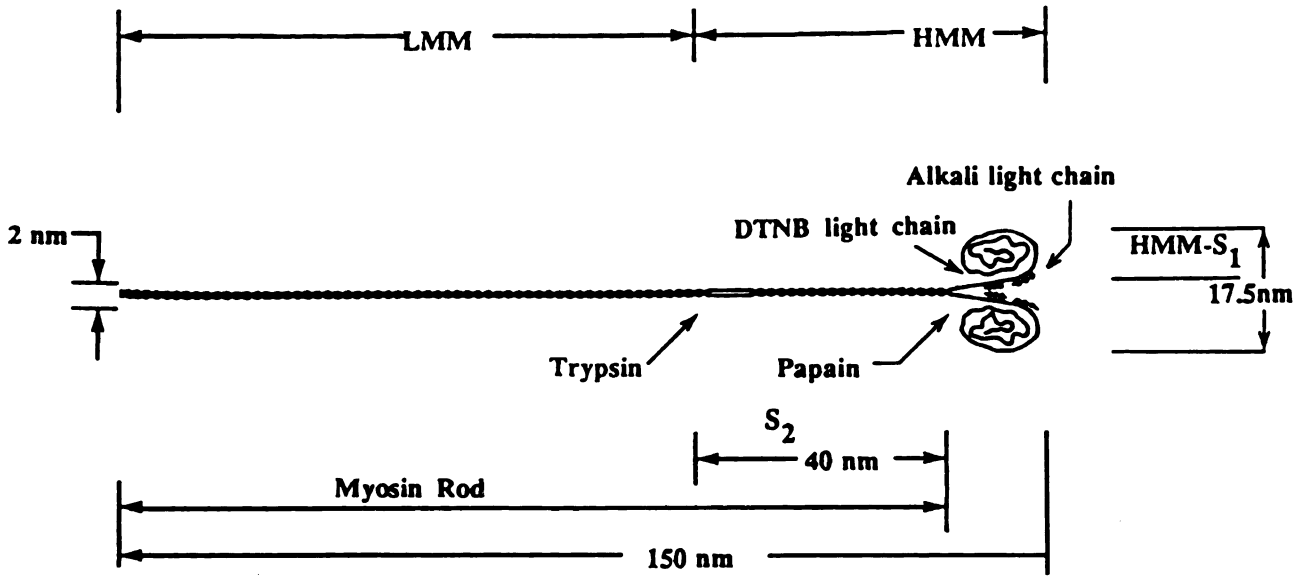

Figure 2.1 Schematic diagram of myosin molecule: HMM, heavy meromyosin (350,000 daltons); LMM, light meromyosin (125,000 daltons); HMM-S_1, myosin subfragment-1 (115,000 daltons); S2, myosin subfragment-2; DTNB, 5,5-dithiobis-(2-nitrobenzoate) (adapted from Smith et al., 1983).

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head or myosin neck. These have been identified as alkali light chains (two types, A1 and A2) and DTNB [5,5-dithiobis-(2-nitrobenzoate)] light chains and can be isolated using alkali or DTNB, a sulfhydryl blocking agent, respectively. The light chains are referred to as either A1, DTNB, and A2 or LC1, LC2, and LC3 listed in order of decreasing molecular weight.

The alkali light chains (25,000 and 16,000 daltons) modulate ATPase activity and actin-binding ability of myosin; thus, they are considered essential for myosin function (Squire, 1986). The alkali chains are identical in the 141 amino acid residues composing the carboxyl terminus; adjacent to this is a region of 8 residues where A1 and A2 differ by only five amino acids. Also, A1 has 41 additional residues in the NH_2-terminal end (Obinata et al., 1981). The myosin heads can have any combination of alkali light chains; both heads can have A1, A2 or one of each. Both alkali chains are elongated shape proteins with 40% α-helix and 20% ß-structure (Knight and Trinick, 1987; Rayment et al., 1993).

The DTNB light chain has a regulatory function related to myosin contraction. The molecular weight of about 18,000 daltons and the amino acid sequence are very different from the alkali light chains. The DTNB light chain has an elongated shape similar to the alkali light chain, but has a lower fraction of α-helical structure than alkali light chains (Rayment et al., 1993). Two thiol groups are present in the regulatory chain or DTNB light chain in comparison with only one in each essential chain or alkali light chain (Obinata et al., 1981). DTNB has a binding site for single, non-specific divalent metal (Knight and Trinick, 1987), and the capability to be phosphorylated, which modulates muscle

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2.2 MUSCLE FIBERS

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Trypsin and chymotrypsin hydrolyze myosin in the hinge region forming two large fragments called light meromyosin (LMM, 150,000 daltons) and heavy meromyosin (HMM, 350,000 daltons) (Lowey et al., 1969). The HMM retains the actin binding and ATPase activity and is soluble at low ionic strength. The LMM can form thick filaments and is soluble at high ionic strength (Lowey et al., 1969; Obinata et al., 1981). The HMM can be hydrolyzed at the head-tail junction by papain, resulting in the α-helical rod portion (HMM S-2) and head portion (HMM S-1) (Lowey et al., 1969). The HMM S-1 portion possesses both the actin binding and ATPase activity. When the myosin rod is treated with trypsin or chymotrypsin, LMM and HMM S-2 are obtained (Balint et al., 1968).

Proteolytic cleavage of the myosin head produces three separate domains. Starting from the N-terminal end of the heavy chain, these peptides possess molecular weights of 25, 50, and 22 kilodaltons (kDa) (Mornet et al., 1979 and 1981). It has been reported that each domain can be related to a specific function of myosin (Burke et al., 1987; Mornet et al., 1979). The 25 kDa and 50 kDa fragments are related to the ATP binding site (Mahmood and Yount, 1984; Squire, 1986) and the 50 kDA and 22 kDa domains participate in the actin binding site (Yamamoto and Sekine, 1979a,b,c).

2.2 MUSCLE FIBERS

Myosin forms thick filaments through interactions of the rod regions. Thin filaments are mainly composed of actin. Thick and thin filaments interact at the myosin head. The cytoskeletal proteins keep

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myosin and actin in register to form an organized structure called myofilaments. Myofilaments form bundles called myofibrils, which are assembled in muscle fibers (Figure 2.2). Muscle fibers are the structural unit of skeletal muscle tissue and are surrounded by connective tissue called the endomysium. Muscle fibers are gathered into bundles called primary bundles, which are in turn grouped in bundles known as secondary bundles. Primary and secondary bundles are surrounded by perimysium. External connective tissue, epimysium, surrounds the entire muscle.

2.2.1 Muscle fiber origin

Muscle development is schematically illustrated in Figure 2.3. Muscle originates from the mesodermal layer of the embryo through a series of proliferation and differentiation steps (mitosis and quantal mitosis). The mesodermal cells produce the myogenic precursor cells, which then develop into presumptive myoblasts. Presumptive myoblasts are cells which are mononucleated and able to replicate, but cannot fuse or synthesize myofibrillar proteins like the myoblasts. The PMb stop multiplying and develop into a myoblast. The mononucleated myoblasts fuse and synthesize the muscle fiber specific proteins. These proteins are synthesized as different isoforms which are coded from multigene families (Mintz and Baker, 1967; Moore et al., 1992; Muntz, 1990).

There are different types of myoblasts throughout muscle development. The commitment to a specific type causes fiber differentiation by developmental stage (embryo, neonatal or adult) or function (fast, slow or fast/slow contraction). Different studies in chicken and small mammals have found that embryonic, neonatal and adult

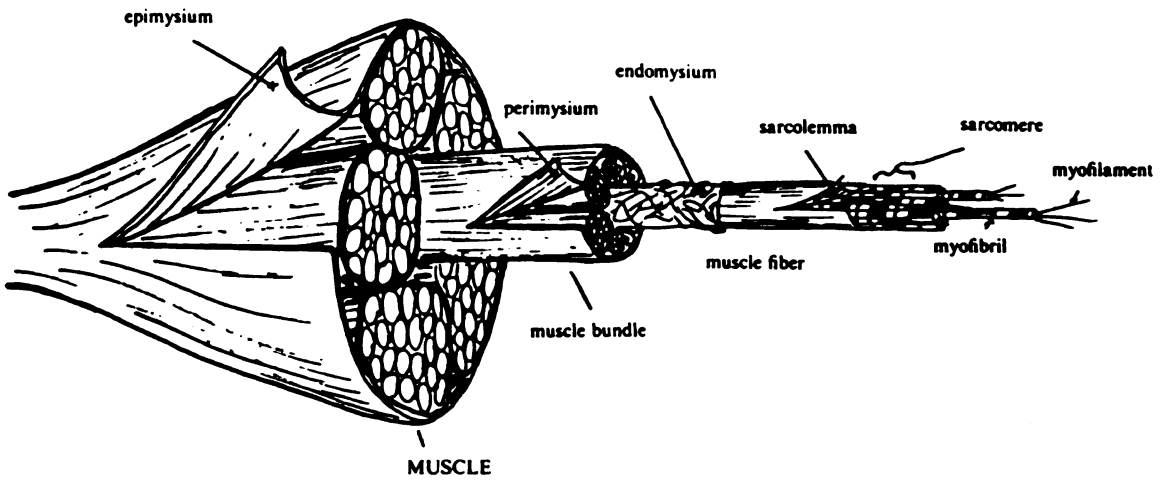

Figure 2.2 Diagram of muscle structure. (adapted from Judge et al., 1989).

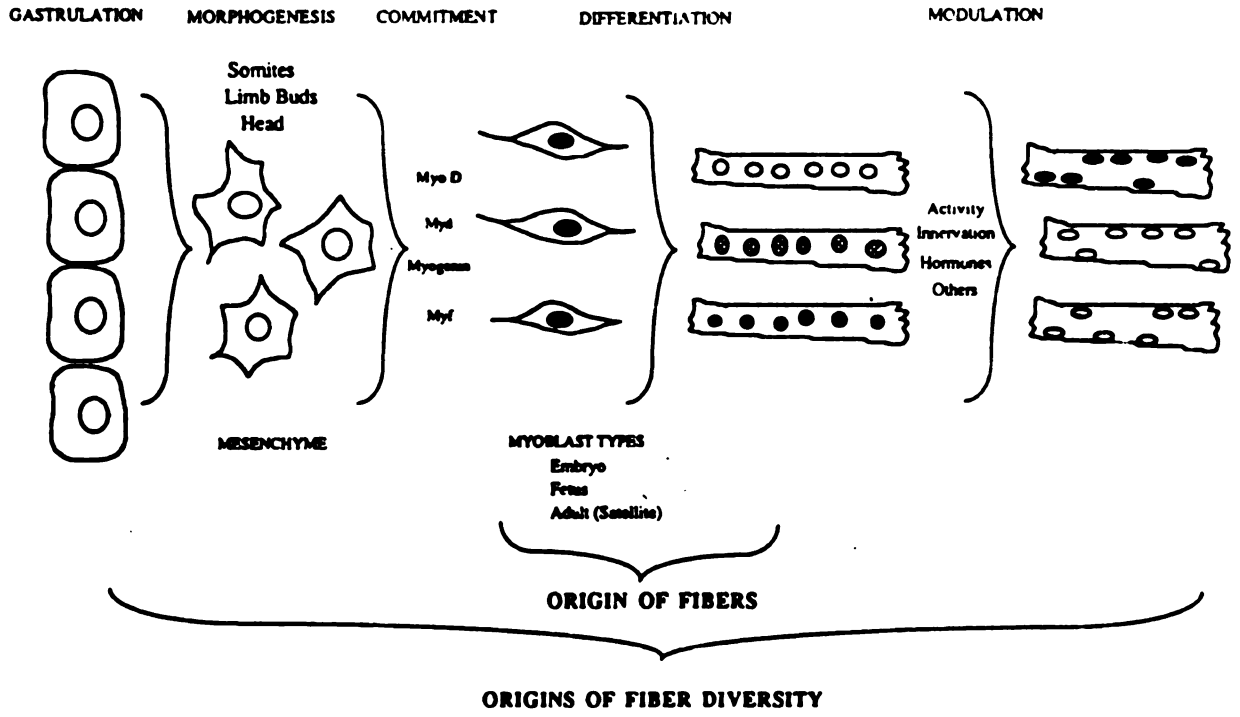

Figure 2.3 Schematic model of muscle development and differentiation (adapted from Stockdale, 1990a).

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forms of myosin are composed of different isoforms (Gauthier et al., 1982, Muntz, 1990; Whalen et al., 1981). Gauthier et al. (1982) stated that these transients do not stop being synthesized, instead a new generation of isozymes replaces the previous stage. There are different factors which affect the final fiber phenotype including activity, innervation and hormones. The appearance of adult myosin isoforms has been related to the development of the neuromuscular system (Muntz, 1990). The definitive fiber type is in a dynamic state and any change in activity, hormones or innervation can modulate the fiber type at any development state (Stockdale, 1990a).

Myotubes, which are multinucleated cells, are produced by fused myoblasts (Mintz and Baker, 1967). Nuclei in the myotube are centrally located and are incapable of mitosis. The mature myotube forms myofibers which are similar to myotubes but with the nuclei located in the cell periphery. The use of monoclonal antibodies to different myosin isoforms provides a tool to study the embryonic origin of muscle fiber types. There are numerous studies in birds (Bandman et al., 1989 and 1990; Gauthier et al., 1982: Hofmann et al., 1988; Maruyama and Kanemaki, 1990; Stockdale, 1990b; Stockdale and Miller, 1989; Stockdale et al., 1989) that suggest six different myosin heavy chain isoforms are synthesized during development. Robelin et al. (1993) studied the development of myosin in semitendinosus muscle of cattle from 39 days of gestation to 30 days after birth using four different antibodies. The authors found that there were two generations of cells: the first cells produced only type I myosin, whereas the second generation of cells that appear later, around 120 days of gestation, produced type I and II. In cattle, as in humans, embryonic and fetal myosin are not synthesized

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2.2.2 Muscle Fiber Types

The terms, muscle type and fiber type, are used interchangeably in the literature, but muscles actually contain a mixture of fiber types. A muscle containing 40% or greater of a fiber type can be classified as white or red muscle (Beecher et al., 1965). There is extensive information about muscle fiber types in skeletal muscle (Brooke and Kaiser, 1970; Cassens and Cooper, 1971; Gauthier, 1970; Salviati et al., 1982; Sams and Janky, 1990; Seideman and Crouse, 1986; Solomon and Montgomery, 1988; Solomon et al., 1985; Suzuki and Cassens, 1980; Totland et al., 1988). Methods of classification are based on specific characteristics of each fiber type: appearance, physiological behavior, biochemical properties or histochemical staining properties (Cassens and Cooper, 1971).

The most accepted method of classification is to divide fibers into three types known as type I, type IIa and type IIb based on three features: speed of contraction, glycolytic capacity and oxidative capacity (Peter et al., 1972). A fiber type present in small quantities in some muscle, type IIc, is thought to be a neonatal fiber in transition to the other types (Cleveland et al., 1977).

Type I fibers or ß-red are slow contracting and have a predominantly oxidative metabolism (Brooke and Kaiser, 1970). Red fibers tend to be small in size, contain high numbers of mitochondria which can be located at the Z-band and in the interfibrillar space (Gauthier, 1970), and have a high content of lipid and myoglobin. The Z-band is wider in red fibers than in white fibers (Gauthier, 1970).

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Type I fibers are more resistant to fatigue due to their slow rate of ATP hydrolysis (Beatty and Bocek, 1970). The blood supply to type I fibers is more abundant and fibers contain more RNA with increased ability to synthesize protein. Red fibers react strongly with oxidative stains such as succinate dehydrogenase (SDH); nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) and myosin adenosine triphosphatase (ATPase) under acid conditions during histochemical staining.

Type IIb fibers or α-white have a fast rate of contraction and relaxation which can be related to the more abundant and better developed sarcoplasmic reticulum (Pearson and Young, 1989). White fibers are generally larger than red fibers. Type IIb fibers contain larger amounts of glycogen compared with red fibers and glycolytic metabolism predominates (Pearson and Young, 1989). In the case of histochemical staining, white fibers react strongly with glycolytic stains, such as myosin ATPase under alkaline conditions and amylophosphorylase (AP).

Type IIa fibers or α-red have intermediate properties with mixed oxidative and glycolytic metabolism. Intermediate fibers are similar to white fibers, although they have slightly slower intrinsic rates of shortening (Pearson and Young, 1989). They contain a large number of mitochondria and thus are more resistant to fatigue. The Z-lines in the intermediate fibers is thinner than the red fiber and this is one feature which can be used to distinguish red and intermediate fibers.

2.3 MUSCLE PROTEIN ISOFORMS

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fiber types (Gauthier and Lowey, 1979; Schiaffino et al., 1990). There are numerous possible combinations of myosin isoforms due to the hexameric nature of myosin (Staron and Pette, 1990). Myosin heavy chains have been suggested to be the major contributor to myosin diversity (Moore et al., 1992). The myosin heavy chains from slow muscle can be combined with slow or fast myosin light chains. Young (1982), showed with peptide maps, that there are differences in myosin heavy chains of fiber types I and II. In slow myosin, only one heavy chain polypeptide was found per species (Billeter et al., 1981; Salviati et al., 1982; Young and Davey, 1981). Two different maps were obtained of purified single bovine fast fibers which were different from the slow heavy chain and from each other (Young and Davey, 1981). Later, Young (1982) established that each variant is predominant in each of the two type II fiber types. Using gradient sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), myosin heavy chain (MHC) isoforms have mobilities from fastest to slowest of: MHCIIa, MHCIIb, and MHCI (Bär and Pette, 1988; Carraro and Catani, 1983; Sugiura and Murakami, 1990; Termin et al., 1989). Moreover, these protein bands have been identified using monoclonal antibodies for each specific myosin heavy chain by Western blot and enzyme-linked immunosorbent assay (Betto et al., 1986; Lowey, 1980; Picard et al., 1994).

Myosin light chains vary with fiber type (Lowey and Risby, 1971). In contrast to myosin heavy chain isoforms which have similar molecular weights (Young and Davey, 1981), myosin light chain molecular mass varies between fiber types (Sarkar et al., 1971). In the type IIb or fast-twitch fibers, three different myosin light chains have been identified: LC1f, LC2f and LC3f. LC1f and LC3f are alkaline light

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chains with molecular weights of 25 and 15 kDa, respectively. LC2f, corresponding to DTNB light chain, has a molecular weight of 17 kDa (Sarkar et al., 1971). The ratio of LC1f to LC3f in the fast muscle is 2:1 (Asghar et al., 1985).

Slow or red fibers contain two types of light chains, LC1s and LC2s, which are similar to cardiac LC1 and LC2. The LC1s has a higher molecular weight than LC1f. LC1s can be either of two different polypeptides - LC1sa and LC1sb - with molecular weights of 27 and 26 kDa, respectively (Sarkar et al., 1971; Weeds, 1976 and 1980). The LC2s has a molecular weight of 19 kDa and is similar to LC2f. The ratio of LC1s to LC2s is 1:1 for myosin head. The stoichiometry between LC1sa and LC1sb is unknown.

Actin does not differ with skeletal muscle fiber type (Pearson and Young, 1989) and is called α-actin. This type is also present in cardiac muscle and small percent of cardiac α-actin. Tropomyosin is composed of α and ß chains. For fast skeletal muscle, the α:ß ratio is 3-4:1 and for slow skeletal muscle the ratio is 1:1 (Cummins and Perry, 1974; Obinata et al., 1981). The difference in the α:ß ratio between fiber types has been related to differences in troponin T binding and some glycolytic enzymes (King and Macfarlane, 1987). The three troponin subunits (I, C, and T) exist as slow and fast isoforms (Dhoot et al., 1979; Hartner and Pette, 1990; Salviati et al., 1982). C-protein binds myosin and is a common impurity during myosin purification (Starr and Offer, 1971). C-protein fast and slow isoforms have been identified in mammals and birds, and have slightly different mobilities during SDS-PAGE (Callaway and Bechtel, 1981). Xiong et al. (1987) observed more intense bands around 300 kDa molecular weight in electrophoretograms of

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chicken white muscle fiber proteins than red muscle, which were tentatively identified as connectin, nebulin and filamin (Bechtel, 1979; Elgasimi et al., 1985; Wang, 1981).

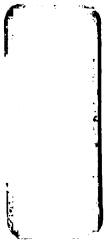
2.4 PROTEIN GELATION

Thermal gelation of meat proteins is the most important functional property determining textural characteristic of processed meat products (Acton and Dick, 1989; Foegeding, 1988; Smith, 1988).

2.4.1 Theory and mechanism

Protein gels have been described as "three-dimensional matrices or networks in which polymer-polymer and polymer-solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water by a small proportion of protein" (Flory, 1974; Hermansson, 1979; Mulvihill and Kinsella, 1987; Morrissey et al., 1987).

The formation of a protein gel has been suggested to follow a two step mechanism involving unfolding of protein (denaturation) followed by an aggregation process (Ferry, 1948). Foegeding and Hamann (1992) presented a model of heat-induced gelation (Figure 2.4): proteins unfold, aggregate, and when the gel point is reached, start to form a three-dimensional network with viscoelastic properties. Denaturation of protein involves a change in the secondary, tertiary, or quaternary structure without changing the amino acid sequence (Tanford, 1968). Protein unfolding, in this case due to an increase in temperature, can be measured by analytical methods which detect any change in the structure of the native protein molecule such as differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy, Raman



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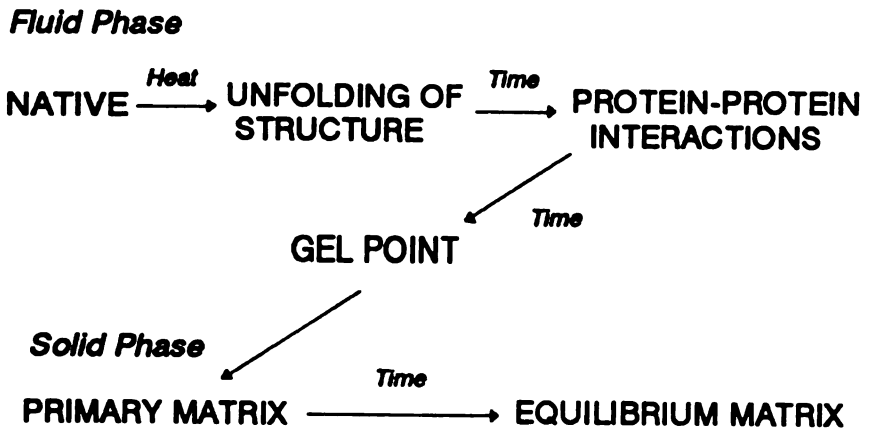

Figure 2.4 Schematic model of thermally induced gelation of protein (adapted from Foegeding and Hamann, 1992).

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spectroscopy, circular dichroism, and monoclonal antibodies. Clark and Lee-Tuffnell (1986) stated that, in some cases, only partial unfolding of protein is necessary to initiate protein gelation. When temperature is increased, the Van der Waals interactions between nonpolar residues and hydrogen bonds are stronger than the hydrophobic interactions. Protein stability decreases and the protein unfolds exposing the hydrophobic residues (Nakai and Li-Chan, 1988).

Mulvihill and Kinsella (1987) proposed that the ability of denatured protein to associate and coagulate, precipitate, or gel depends on the protein, its amino acid composition, molecular weight, net hydrophobicity, concentration, and critical balance between attractive and repulsive forces. The aggregation of unfolded proteins is due to protein-protein interactions. Turbidity, light scattering, or other forms of spectroscopy can be used to follow protein aggregation (Hermansson, 1979; Smith, 1994). Protein aggregation continues until a critical point of crosslinking is reached; then a three-dimensional network, a gel, is formed through a competition of attractive forces of unfolded protein and the repulsive forces due to protein charge (Clark et al., 1981). Dynamic testing has been suggested as the best method to study the formation of the gel network (Clark, 1992). During gelation, the protein solution forms a viscoelastic solid; small strain dynamic testing is used to monitor this transition given that this method will not disrupt the gel matrix formed (Smith, 1994; Steffe, 1992). Other methods measure the characteristics of the final gel network such as texture profile analysis (Bourne, 1978; Mittal et al., 1992); compression tests (Lee and Chung, 1989) and torsion failure tests (Montejano et al., 1985) where strain-stress at gel structural breakdown

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2.4.2 Methods to follow gelation

The principle and parameters measured by the three methods used in this study to follow protein gelation will be described in this section.

2.4.2.1 Differential Scanning Calorimetry (DSC)

Calorimetry can be used to measure the thermodynamic parameters related to structural changes of protein and other macromolecules with systematic variations in temperature (Donovan, 1984; Krishnan and Brandts, 1978; Ma and Harwalkar, 1991; Stabursvik and Martens, 1990). DSC is used to measure changes in heat capacity (C_p, cal/mol K) as a function of temperature. C_p is defined as the amount of heat needed to increase one mol of substance 1°C and can be calculated as a derivative of enthalpy with respect to temperature at constant pressure. During a DSC run, protein structural changes are recorded as peaks in the heat capacity curve or thermogram. The temperature where 50% of the molecule has been denatured is called the melting temperature (T_m). The calorimetric enthalpy (ΔH_{cal}, cal/mol) is defined as the area under the C_p curve as a function of temperature and can be calculated by:

$$\Delta H_{cal} = \int \Delta C_p dT$$

where C_p is heat capacity and T temperature.

If protein unfolding is considered as two thermodynamically stable states, native and denatured, an equilibrium thermodynamic constant can be calculated. The van't Hoff enthalpy (ΔH_{vH}, cal/mol) can be calculated by the temperature variation of the equilibrium constant and

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$$\Delta H_{vH} = \frac{4RT_m^2 C_{p(max)}}{\Delta H_{cal}}$$

Large proteins usually fold in smaller subunits which have a stable tertiary structure, called domains (Bertazzon and Tsong, 1989). The ratio of van't Hoff enthalpy to the calorimetric enthalpy is defined as the cooperativity ratio (CR). Tsong et al. (1970) indicated the following relationship between CR and protein domains:

CR > 1 multidomain protein

CR = 0 a two state unfolding process or single domain

CR < 1 aggregation or intermolecular interactions

The heat capacity endotherm can be deconvoluted into several transitions corresponding to the unfolding of each domain. These are assumed to be independent two-state transitions with $\Delta H_{cal} = \Delta H_{vH}$. A least squares fitting method was used (Freire and Biltonen 1978a,b; Ramsay and Freire, 1990) to determine transition temperature (T_n) and enthalpy of each domain.

2.4.2.2 Turbidity

The optical density of a protein solution increases during heating due to the change in size and number of particles. As proteins unfold, they interact with other protein molecules and form aggregates via covalent or non-covalent bonds, and the amount of light that passes through the solution is decreased (Clark and Ross-Murphy, 1987). Hermansson (1979) and Ferry (1948) found that the rate of aggregation must be slower than denaturation to allow the denatured protein to

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orient and interact in orderly manner to form an orderly network (gel). Moreover, the final characteristics of the gel network depend on the aggregation rate of the denatured protein; a slower rate results in a finer gel network (Hermansson, 1978).

The changes in optical density (OD) as a function of temperature can be used to find the initial temperature of aggregation (T_o), defined as the temperature where the optical density starts to increase significantly (at least 0.02) from the initial OD. Typically, most turbidity tests are designed to follow the increase of absorbance or OD with temperature (Ndi and Brekke, 1992; Samejima et al., 1989; Xiong and Blanchard, 1994; Xiong and Brekke, 1990a). The derivative of absorbance with respect to temperature (dA/dT) (Robe and Xiong, 1992 and 1994; Xiong and Brekke, 1990b) is used to determine the temperature where transitions or inflection points occur during heating. Another possible way to interpret turbidity is to adjust the result to a sigmoidal curve to obtain the rate of protein aggregation measured by a derivative of OD with respect to temperature (Liu et al., 1995).

2.4.2.3 Dynamic rheological testing

During gelation, the protein sol changes from a viscous liquid to a viscoelastic solid. When a viscoelastic material is subject to a constant stress, part of the energy is dissipated as heat (viscous part) and part is stored (elastic element). The material may partially recover when the stress is removed. In a viscoelastic material, when sinusoidal oscillating stress is applied, the resultant strain will be out of phase between 0° and 90°.

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and the resultant stress or strain on the sample is measured (Murayama, 1978) using a cone and plate or parallel plate apparatus. If the resultant harmonic stress amplitude is proportional to the applied strain amplitude with a phase lag with respect to the strain, then the stress will be independent of the strain and the material is assumed to deform linearly (linear viscoelasticity). When a harmonic strain with amplitude γ_0 and frequency ω (radians/s) is applied to the upper plate, the resultant stress can be used to measure material properties. The following terms can be used to describe linear viscoelastic behavior:

Applied strain (dimensionless) $\gamma = \gamma_0 \cos \omega t$

Output stress (Pa) $\sigma = \sigma_0 \sin (\omega t + \delta)$

Storage modulus (Pa) $G' = (\sigma_0 \cos \delta) / \gamma_0$

Loss modulus (Pa) $G'' = (\sigma_0 \sin \delta) / \gamma_0$

Complex modulus (Pa) $G^* = (G'^2 + G''^2)^{1/2}$

Tangent delta $\tan \delta = G''/ G'$

where γ_0 is the amplitude of strain function

σ_0 is the amplitude of stress function

t is time

δ is the phase lag

Storage modulus (G') is a measurement of the solid behavior of a sample. As protein gel crosslinking increases, the magnitude of G' will increase. The loss modulus (G"), an indication of the viscous behavior of the gel, will decrease as the gelation reaction progresses. The transition from sol to a gel can be monitored by the change in tan δ (Hamann, 1991). Tan δ decreases as the gel is formed. G' and G" are dependent on frequency. Low frequencies (ca. 1 Hz) should be used to more accurately detect the gel point (Clark, 1992).

2.4.2.4 Gel structure evaluation

Large deformation or destructive studies have been used to evaluate the structure of a gel matrix. In this study, three methods were used to measure the gel characteristics of salt soluble proteins: texture profile analysis (TPA), apparent strain and stress at failure by compression testing (Nuckles et al., 1991); and stress and strain at failure by torsion analysis (Amato et al., 1989; Foegeding, 1990; Montejano et al., 1985). TPA has been used to measure meat texture (Berry, 1983; Mittal et al., 1992; Saliba et al., 1987). Bourne (1978) discussed the definition and calculation of TPA parameters: brittleness, hardness, springiness, cohesiveness, adhesiveness, gumminess and chewiness.

Apparent strain and stress at failure are calculated when the gel is subjected to uniaxial compression between two parallel plates. Different studies have shown good correlations with sensory data, such as firmness and smoothness (Chai et al., 1991; Chung and Lee, 1990; Huang and Robertson, 1977). Stress at failure indicates firmness or hardness of a gel and strain at failure is an indicator of gel cohesiveness (Hamann et al., 1987). One problem with this type of measurement is that a gel may not fail.

In the torsion technique, the gel is shaped into a known geometry, most commonly a capstan, then twisted in a viscometer at a pre-determined rpm and resultant torque measurements are obtained. Diehl et al. (1979) derived the true shear stress (TSS, kPa) and true shear strain (γ) using the torque and time data from torsion experiments. Montejano et al. (1985) compared this technique to TPA using 8 different protein gels and found that TSS and γ correlated with hardness and

cohesiveness. The torsion test has some advantages compared with the other techniques: 1) no visible change occurs in gel volume, 2) highly elastic gels will fail, and 3) given that tensile, compressive, and shear stress are equal, the weaker of any of those stresses will result in gel failure (Montejano et al., 1985).

2.5 GELATION OF SALT SOLUBLE PROTEINS

Proteins from the myofibrillar fraction have been found to be most responsible for meat protein gelation (Choe et al., 1991). Fukazawa et al. (1961a,b) studied the role of different myofibrillar proteins in texture formation during sausage manufacture. Among the myofibrillar proteins, myosin was mainly responsible for gel properties (Fukazawa et al., 1961a,b; Samejima et al., 1969; Yasui et al., 1982). The form of myosin, free or as actomyosin, resulted in different gel strengths (Asghar et al., 1985). Actin does not gel, but might influence myosin gelation (Samejima et al., 1969; Wang and Smith, 1994b). This influence was suggested to be ionic strength dependent (Asghar et al., 1985). Later, Wang and Smith (1994a) found that the presence of actin affected the denaturation of structural domains of myosin, altering its gelation. Asghar et al. (1985) postulated that the ratio of myosin/actomyosin has a greater influence on gel strength than myosin/actin ratio. Addition of troponin and tropomyosin did not enhance myosin gel strength or microstructure (Samejima et al., 1982).

Sarcoplasmic and stromal proteins can modify the gelling properties of SSP gels. Nuckles et al. (1991) stated that when 8.33% of the myofibrillar protein was replaced by the sarcoplasmic protein fraction, gel strength of beef semitendinosus muscle decreased. Acton

et al. (1983) suggested that sarcoplasmic proteins may interfere with SSP cross-linking during matrix formation of gels. Substitution of stromal proteins at 8.33% did not affect gel hardness and deformability of beef semitendinosus SSP, but at higher substitutions the decreased gel properties (Nuckles et al., 1991).

2.6 MYOSIN GELATION

Given that myosin is mainly responsible for myofibrillar protein gelation, the mechanism of how myosin forms a gel has been investigated in model systems. Monitoring myosin gelation by dynamic testing has determined the role of myosin subunits and transition temperatures for these events. The first event, according to the literature, is the unfolding of HMM reported to start around 37°C (Burke et al., 1987); although, these structural changes had been reported at lower temperatures (ca. 30°C for rabbit) (Yasui et al., 1979). Self-association of myosin filaments through head-to-head interactions to form aggregates similar to "daisy wheels" have been reported to occur up to 40°C for bovine longissimus dorsi (white muscle) (Egelandsdal et al., 1986; Yamamoto, 1990). Using scanning electron microscopy, Sharp and Offer (1992) saw the same type of "daisy wheel" structure for rabbit leg and back muscle myosin after heating to 35°C for 30 min. Denaturation of myosin HMM occurred between 40 to 50°C where the daisy wheel structures started to interact. Between 40 to 50°C, G' and G" increase due to interactions between fish myosin rods (Sano et al., 1988 and 1990). Between 50 to 60°C these oligomers start to aggregate due to the cross-linking of tail regions (Sharp and Offer, 1992). Egelandsdal et al. (1986) stated that the decrease in storage modulus between 50 to

60°C is the result of LMM denaturation and suggested that LMM domains are the first to denature based on Wright and Wilding's results in rabbit (1984). Morrissey et al. (1987) concluded that denaturation appears to involve three regions of the myosin molecule: helical tail, hinge region, and the globular head.

Other factors influence myosin gelation besides the other myofibrillar proteins. Myosin gel strength increases proportionally to myosin concentration (Ishioroshi et al., 1979; Yasui et al., 1982). However, the response of the storage modulus to concentration differs between muscle fiber types. The increase of G' is proportional to the square of myosin concentration from white fibers and close to 1.6 for red fiber types (Fretheim et al., 1986). Two factors which are interrelated and shown to be very significant to the type of gel formed are pH and ionic strength. The type of gel formed is a result of the balance between the attractive and repulsive forces of protein molecules, and this balance depends on the pH and ionic environment (Ziegler and Foegeding, 1990). Hermansson et al. (1986) showed that bovine semimembranosus myosin forms two types of gels depending on the ionic strength of the solution. At low ionic strength (0.1 - 0.2 M KCl and pH 6.0), myosin formed thick filaments and on heating, a finer gel matrix was obtained. When the ionic strength was increased to 0.6 M KCl at the same pH, myosin is monomeric and on heating, myosin aggregates by head-to-head interactions producing coarser gels with weaker matrix (Morrissey et al., 1987).

The effect of pH from 5 to 8 on myosin gelation has been the subject of different studies (Egelandsdal et al., 1994; Ishioroshi et al., 1979; Wang et al., 1990; Yasui et al., 1980). The pH affects the

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conformation and charge distribution on the surface of the protein and subsequently affects denaturation, aggregation, and gel characteristics during heat-induced gelation. DSC studies with rabbit myosin showed that increasing the pH (5.4 to 7.0), decreased the transition temperature (37 to 47°C) (Goodno and Swenson, 1975a,b). An alteration of charge on myosin molecule and a change in the electrostatic forces were suggested by the authors as possible reasons for different thermal behavior with pH. Xiong (1992) suggested that the protein-protein interaction mechanism changes with pH and influences the aggregation rate, which Hermansson et al. (1986) stated leads to differences in gel network formation.

2.7 FIBER TYPE EFFECT IN MYOSIN GELATION

There are extensive data in the literature about variations in gelling properties of red and white muscles or fibers. Results are difficult to compare because research was done in different protein systems and under different environmental conditions.

Poultry species are often used to study the effect of muscle type on gelation, as breast muscle has predominantly white fibers and leg or thigh muscle mostly red fibers (Sams and Janky, 1990). Bovine muscles do not contain pure red or white fibers. Cassens and Cooper (1971) suggested a muscle must contain greater than 40% of a particular fiber type to be classified as white or red muscle. In bovine species, the muscles of choice have been masseter (red fiber) and cutaneus trunci (white fiber), because these muscle can be removed from the carcass without damaging their commercial value (Egelandsdal et al., 1994; Fretheim et al., 1986; Young et al., 1992). Cassens and Cooper (1971)

also pointed out that fiber type composition is related to the pale, soft, and exudative (PSE) condition. PSE pork contains muscle with larger, in diameter, white fibers (Essén-Gustavsson et al., 1992).

One of the first suggested reasons for differences in gelation properties of fiber types was the difference in ultimate pH (Amato et al., 1989). Red muscle has a higher ultimate pH than white muscle (Angel and Weinberg, 1981) in several different species. Chicken gastrocnemius (red fiber) and pectoralis profundus (white fiber) myosin formed stronger gels at pH 5.9 and 5.6, respectively (Asghar et al., 1984). Acton et al. (1983) explained that for a given concentration of actin and myosin, there is an optimal pH for gelation. Some studies have suggested that by combining different pHs and ionic strengths, the gelling properties of red fibers can be improved to resemble white fiber properties (Asghar et al., 1984; Barbut and Mittal, 1993; Morita et al., 1987; Xiong and Blanchard, 1994b). These studies support the data of Young et al. (1992) that the pH where SSP gel texture changes from granular and brittle to smooth and elastic was higher for red muscle (masseter) than white muscle (cutaneus trunci).

2.7.1 Studies using salt soluble proteins

In chicken and turkey, breast SSP (white muscle type) formed more rigid gels and stronger gels to penetration test than thigh or leg SSP (red muscle type) (Dudziak et al., 1988; Foegeding, 1987; Xiong and Brekke, 1990a,b and 1991). The viscoelastic properties of chicken pectoralis major and pectoralis minor SSP were more elastic and less sensitive to changes in pH than those for thigh or drumstick SSP (Xiong and Blanchard, 1994b,c). Amato et al. (1989), using 12% protein meat

batter from turkey and chicken thigh and breast, found that thigh gels had a higher shear stress and strain than breast gels when measured using torsion testing. The pH of the meat batter from each muscle was not adjusted to a common pH (uncooked breast batter, pH 5.5, and thigh batter, pH 6.07). Barbut and Mittal (1993) showed the same results when turkey breast and thigh meat were used at their ultimate pH (pH 5.8 and 6.5, respectively). However, when turkey breast was adjusted to thigh ultimate pH, both muscles had similar water holding capacity and hardness during TPA. However, turkey thigh at pH 5.8 had higher water holding capacity and hardness than breast at the same pH, but lower than its water holding at pH 6.5.

At the same pH, myofibrillar protein from bovine cutaneus trunci had a lower gelation temperature (ca. 10°C) and formed more rigid gel than masseter using a thermal scanning monitor (Young et al., 1992). The same muscles were used by Meyer and Egelandsdal (1992). They observed a higher complex modulus for cutaneus trunci than masseter after heating, using whole and comminuted muscle in presence of 0.95 and 2.5% NaCl.

Thermally induced denaturation from chicken postrigor breast (white) and leg (red) SSP were slightly different at 0.6M NaCl. Lower transition temperature (ca. 1°C) was observed for leg than breast SSP at pH 5.50 and 6.0 and 1.8°C higher transition temperature for breast SSP at pH 6.05 (Xiong and Brekke, 1990b).

When protein-protein interactions were measured by turbidity, chicken breast SSP at 0.6M NaCl pH 6.0 had a lower onset temperature (0.02 OD change from initial value) and slower rate of aggregation than those for leg SSP during heating (Xiong and Brekke, 1990c). Similar

trends in turbidity were reported using porcine longissimus dorsi (75% white fiber) and vastus intermedius (76% red fiber) in 0.6M NaCl and 50 mM Pipes during isothermal heating at different temperatures for 20 min. (Robe and Xiong, 1994). The rate of aggregation for chicken breast and leg followed first-order kinetics (Acton et al., 1981; Xiong and Brekke, 1990c). However, differences in the slope of the Arrhenius plot of maximum aggregation rate suggested different reaction mechanisms between fiber types (Xiong and Brekke, 1990c). These findings were confirmed later by Ndi and Brekke (1992) for SSP from duck breast (mix of red and white) and leg (red) at pH 5.5, 5.75 and 6.0. Duck breast SSP showed an intermediate behavior of aggregation rate between chicken breast and leg.

Turkey breast SSP had higher water holding capacity than turkey thigh SSP at pH 7.0 and 0.5 M NaCl (Foegeding, 1987). When the pH was decreased to 5.0, the SSP from both muscles did not yield stable gels (Foegeding, 1987). Chicken breast SSP had higher moisture loss than thigh SSP gel in both the pre-rigor and post-rigor state at 0.6M NaCl and pH 6.0 (Xiong and Brekke, 1990b). Angel and Weinberg (1981) found that chicken leg SSP formed gels at lower concentrations than breast SSP at pH 6.2 and 5.8 determined by least concentration end-point.

2.7.2 Studies using myosin

Asghar et al. (1984) obtained a higher gel rigidity for chicken pectoralis profundus (white) myosin than gastrocnemius (red) myosin in 0.6M NaCl and 0.02M phosphate buffer, pH 6.0, after heating at 65°C for 20 min. Chicken pectoralis (white fiber) myosin had a lower onset temperature for gelation and higher storage modulus at the end of the

heating period (70°C) than myosin from ilictiobialis and gastrocnemius (red fiber) in 0.6M NaCl and pH 6.0 (Liu, 1994). The storage modulus during heating of bovine cutaneus trunci (white muscle type) myosin was higher than those from masseter (red muscle type) at 0.2 and 0.6M NaCl and four pHs: 5.5, 5.75, 6.0 and 7.0 (Egelandsdal et al., 1994). Fretheim et al. (1986) found the same behavior using the same muscle, but at pH <5.8, masseter presented higher storage modulus than cutaneus trunci.

Differences in the thermal transitions of myosin isoforms were suggested as one possible reason for the different behavior. Using DSC, Liu et al. (1995) found that chicken pectoralis (white fiber) myosin underwent the first transition at 48.1°C. The Tms of iliotibialis and gastrocnemius (red fiber) myosins were 49.5 and 49.3°C, respectively. The ΔH_{cal} was higher for pectoralis than for the other two muscles. However, Dudziak et al. (1988) found the same thermal transitions for turkey breast and thigh myosin, although actomyosin gels with different rheological properties were obtained. Bovine cutaneus trunci myosin had a lower enthalpy of denaturation compared with bovine masseter myosin (Egelandsdal et al., 1994). In this study, thermograms of masseter and cutaneus trunci myosin showed different peak separations at pH 5.5, 6.0 and 7.0, and two salt concentrations (0.2 and 0.6M NaCl). Lowering the pH decreased apparent enthalpy of denaturation in both muscles and the effect was greater in cutaneus trunci than masseter. The transition temperature was higher for masseter than cutaneus trunci. The authors suggested that cutaneus trunci was less heat stable than masseter. In 0.6M NaCl, myosins of both muscles showed two distinct peaks. The first transition has been assigned to denaturation of the second part of the

myosin rod overlapping with head denaturation (Samejima et al., 1983). The second transition is due to denaturation of the rod. The last transition appeared at a higher temperature for cutaneus trunci than masseter, suggesting than this part of the rod is more stable in cutaneus trunci (Egelandsdal et al., 1994).

The aggregation of myosin from different muscle types has been studied as a function of time. Chicken pectoralis myosin began to aggregate after 5 or 6 minutes of heating at 45°C ,while iliotibialis and gastrocnemius needed to be heated to at least 50°C for aggregation to occur (Liu et al., 1995). Using derivative curves of protein-protein interaction for chicken breast and thigh actomyosin, two distinct transition temperatures were observed. The first transition temperature occurred at a lower temperature and the second at a higher temperature for breast (49.2 and 60.2°C) than those for thigh (52.6 and 57.9°C) (Acton and Dick, 1986). These results suggested that less energy is required for thermal aggregation of thigh actomyosin compared with breast actomyosin. Ziegler and Foegeding (1990) suggested, based on these results, that the difference came from variation in the aggregation step of heat-induced gelation. Egelandsdal et al. (1994) followed the filament formation of bovine cutaneus trunci and masseter after 3 and 20 hrs of preparation of myosin solution from their $(NH_4)_2SO_4$ salts by turbidity at 0.1 to 0.6M NaCl between pH 5.50 to 7.0. Higher ODs were obtained in general for cutaneus trunci than masseter.

2.7.3 Studies with mixed fibers or myosin subfragments

The interaction between red and white SSP or myosin systems might have a synergistic effect on gel properties. A higher storage modulus

was obtained when 25, 50, and 75% of white fiber was substituted for red fiber than those obtained with 100% of red or white fiber at a final concentration of 10 mg/mL in 0.6M NaCl and pH 5.65 and 6.0 (Choe et al., 1991; Fretheim et al., 1986).

Several researchers have studied myosin and myosin subfragment gel properties from chicken thigh and breast (Asghar et al., 1984; Choe et al., 1989 and 1991; Chung-Wu, 1969; Samejima et al., 1989). Choe et al. (1989 and 1991) showed that differences in filament forming ability of myosin rod may cause differences in gel strength. Different thermogelation behavior of myosin from different fiber types was suggested as a cause for differences in filamentogenesis.

CHAPTER 3

COMPOSITION, PH SOLUBILITY PROFILE, AND THERMAL GELATION OF SALT SOLUBLE PROTEINS AS INFLUENCED BY BOVINE MUSCLE TYPE.

3.1 ABSTRACT

Heat-induced gelation of muscle salt soluble proteins (SSP) contribute to the yield and textural properties of processed meat products; however, the influence of bovine muscle fiber type on the thermal gelation properties of these proteins is poorly understood. The purpose of this study was to compare the composition, pH solubility profile and thermal gelation behavior of two bovine muscles, vastus intermedius (VI, predominately red fibers) and semimembranosus (SM, predominantly white fibers). Muscle composition and ultimate pH were determined. SSP protein extracts were used to compare solubility profiles, dynamic rheological properties during heating and texture properties of heat-induced gels. VI had a higher fat content and lower protein content than SM. Ultimate pH was 6.0 for VI and 5.5 for SM. Solubility of VI SSP proteins decreased by 30% ($p<0.05$) between pH 5.8 and 5.6, whereas solubility of SM SSP decreased ($p<0.05$) by 30% between pH 5.6 and 5.4. Torsion and dynamic properties were measured at pH 5.5 and 6.0. Stress and strain at fracture of SSP gels were the same ($p>0.05$) for SM at both pH's and VI at pH 5.5, but higher in VI at pH 6.0. VI gels had higher syneresis and lower expressible moisture than SM. SSP from both muscles had lower syneresis and expressible moisture

at pH 6.0. Dynamic rheological properties of 4% (w/w) SSP solutions heated from 30 to 80°C at 1°C/min varied with pH and muscle type. The storage moduli (G') at 80°C were 1.8 kPa, 1.2 kPa, 1.1 kPa and 0.2 kPa for **SM**-pH 5.6, **VI**-pH 6.0, **SM**-pH 6.0 and **VI**-pH 5.6, respectively. Initial increases in G' of both red and white SSP at pH 5.5 were at a lower temperature (about 36°C) than those at pH 6.0. **VI** SSP formed a more elastic gel network than **SM** SSP as indicated by tan δ; however, tan δ at pH 6.0 in **VI** and **SM** SSP were lower than at pH 5.5. Proteins from white muscles formed harder gels with a less elastic matrix and better water-holding ability than those from red muscle. Some gel properties of red muscle proteins were improved by decreasing the pH to that of white muscle.

3.2 INTRODUCTION

Muscle is classified by fiber type. The most accepted classification is to divide fibers into three types known as type I (also called slow or ß-red) muscle, type IIa (intermediate or α-red), and type IIa (fast or α-white). Red or slow muscle are higher in moisture and fat and lower in protein content (Beechel et al., 1965; Cassens and Cooper, 1971; Marchello et al., 1968; Meyer and Egelandsdal, 1992). Also, muscle types differ in ultimate pH (Breidenstein et al., 1968; Meyer and Egelandsdal, 1992; Ramsbottom and Strandine, 1948). Different isoforms of protein are also present in each muscle type (Cummins and Perry, 1974; Lowey and Risby, 1971; Obinata et al., 1981; Young, 1982; Young and Davey, 1981). These differences might be responsible for the differences between the heat-induced gelation properties of SSP from different muscle types (Xiong, 1994).

The muscle proteins can be divided into three categories depending on their solubility: water or low ionic strength soluble (sarcoplasmic), salt or high ionic strength soluble (salt soluble), and insoluble protein fractions. The most important of the muscle proteins are the myofibrillar or salt soluble proteins (SSP) as they contain the most functional protein in a processed meat product. The salt soluble proteins are comprised primarily of myosin and actin. Myosin is responsible for most of the functional properties of processed meat. Actin does not form a gel, but may affect myosin gelation (Asghar et al., 1985; Wang and Smith, 1994a). The other SSP influence the functional behavior of myosin and actin (Smith, 1988).

The influence of muscle type on the heat-induced gelation of SSP has been studied in poultry, pork, beef and fish. Foegeding (1987) determined that gels from turkey breast SSP (fast muscle) gave higher stress at failure and more stable than those from thigh SSP (slow muscle) at pH 6.0. Barbut and Mittal (1993) studied turkey thigh and breast meat at different ultimate pH's of 6.5 and 5.8, respectively. The modulus of rigidity (G) of breast and thigh meat at their own ultimate pH was higher for thigh than breast. When the pH of breast was adjusted to 6.5, maximum G, hardness and water retention were similar or higher than thigh (Barbut and Mittal, 1993; Daum-Thunberg et al., 1992). Moreover, breast SSP formed a gel at a lower protein concentration than thigh. However, Amato et al. (1989) reported stress, strain and water retention of turkey and chicken breast gels were lower than those from thigh muscle, but comminuted whole muscles were used in the manufacture of these gels. Xiong (1992) studied the thermal behavior of myofibrillar proteins from breast and leg chicken muscles by measuring

the changes in turbidity by optical density during heating. The thermal transitions of leg SSP occurred at higher temperatures than for breast SSP under the same conditions, suggesting that breast SSP were less thermally stable. The author also found that pH had a large influence on the aggregation step of gelation. The number of transitions increased and the transition temperature decreased when pH was increased from pH 5.50 to 7.0. The shear stress of chicken white muscle SSP was greater and the peak temperature was lower than chicken red muscle SSP during heating from 5 to 50°C (1% protein solution; Xiong and Blanchard, 1994a).

Comparison between SSP of different muscle types in beef is more difficult given that there are fewer muscles of specific fiber types and these muscles are less accessible in the carcasses. Young et al. (1992) studied the gelation properties of two bovine muscles: cutaneus trunci (type II or white) and masseter (type I or red) at different pHs. The results of this study followed the same trend as poultry; cutaneus trunci myofibrillar protein formed a more rigid gel than masseter protein at the same pH. Similar trends in rheological properties were observed when whole chopped or ground masseter and cutaneus trunci from Norwegian red cattle were compared unheated and heated at different temperatures by Meyer and Egelandsdal (1992). Also, pH had an influence on the gel structure that ranged from brittle and granular at low pH to elastic and smooth at higher pH. The pH at which this change of structure occurred was lower for myofibrillar from white muscle than red muscle type (Young et al., 1992). As in poultry, the SSP from white muscle formed a gel at a lower temperature than red muscle type (Young et al., 1992). In previous research, protein isoform type and pH have

been suggested as possible reasons for different behavior during heat-induced gelation. The objectives of the present study were:

- to compare the composition and pH solubility profile of two bovine muscle types.
- to compare the effect of muscle type and ultimate pH on thermal gelation behavior of these two muscles.

3.3 MATERIALS AND METHODS

3.3.1 MATERIALS

Semimembranosus (white fiber muscle) and vastus intermedius (red fiber muscle) muscles from the same carcass were obtained from market weight heifers (16 to 24 months old) slaughtered on ten different times at the Michigan State University Meat Laboratory. The muscles were dissected from the carcass within one week of slaughter. Semimembranosus (SM) and vastus intermedius (VI) muscles were stored in Ziploc bags (Gordon Food Service, Inc, Grand Rapids, MI 49501) at 4°C until used within two days.

3.3.2 EXTRACTION OF SALT SOLUBLE PROTEINS

After fat and excessive connective tissues were trimmed, each muscle was ground twice through a Hobart Kitchen Aid Food Grinder (Model K5-A, Troy, OH 45374) using a 4 mm plate. Ground meat (125 g) from each muscle was stored in Whirl-pak bags (Nasco, Fort Atkinson, WI 53538) for proximate analyses and pH determination the same day that muscles were ground. All extraction procedures were completed at 2° to 4°C. Salt-soluble proteins (SSP) of both muscles were extracted simultaneously as described by Nuckles et al. (1990). Buffered

solutions at pH 7.4 were prepared at least the day before extraction and cooled to 2° to 4°C prior to use. For the low salt extraction, a 0.1 M NaCl in 0.05 M sodium phosphate buffer was used. A 0.6 M NaCl in 0.05 M sodium phosphate buffer was used for the high salt extraction. Each ground muscle was blended with four volumes (w/w) of low salt buffer in a Waring Blendor (Model 1120, Winsted, CT 06057) for two periods of 45 sec. This suspension was stirred for 3 hr with a T-Line laboratory stirrer (Talboy Engineering Corp., Emerson, NJ 18801) with 5.1 cm paddle propeller with 3 blades connected to a rheostat (Powerstat, The Superior Electric Company, Bristol, CT 06010) to control speed (avoiding foaming). Suspensions were centrifuged for 15 min at 5,860 x g. The supernatant was decanted and four volumes of low salt buffer were used to resuspend the pellet. The suspension was stirred for 1 hr, centrifuged and the supernatants, containing the sarcoplasmic protein fraction, were combined and used for further analysis.

The pellet obtained after low salt extraction was dissolved with one-third volume (w/w) of 2.4 M NaCl, 0.05 M sodium phosphate buffer to a final concentration of about 0.6 M NaCl. Three volumes of 0.6 M NaCl, 0.05 M sodium phosphate buffer were added and the solution was stirred for 3 hr. The solution was centrifuged at 10,400 x g for 15 min and supernatant collected. The pellet was stirred a second time with two volumes of 0.6 M NaCl, 0.05 M sodium phosphate buffer for 1 hr and the solution was centrifuged. Supernatants, containing the SSP fraction, were combined. The pellet, containing connective tissue and denatured myofibrillar proteins, was discarded.

The supernatant from the high salt extraction was combined with five volumes of cold (2°- 4°C) deionized water to precipitate the SSP

and centrifuged at 20,000 x g for 15 min. Pellets were combined and centrifuged at 20,000 x g for 15 min to concentrate the protein.

The final pellet was divided into two equal portions and solubilized with one-third volume of 2.4 M NaCl, 0.2 M sodium phosphate buffer at the desired muscle pH. One SSP portion was adjusted to the pH of the original muscle, while the other SSP portion was adjusted to the original pH of the other muscle. For example, one SSP fraction from SM muscle was adjusted to pH 5.45 (the original semimembranosus muscle pH) and the other was adjusted to the original pH of the VI muscle (pH 6.05). To obtain the desired pH of SSP solution, the buffer solutions were at least 1 or 2 pH units lower than the desired pH. If necessary, final pH adjustments were performed using 0.1 M HCl or 0.1 M NaOH solutions.

Protein solutions were held overnight at 2° to 4°C in beakers covered with parafilm and aluminum foil surrounded with ice. Protein concentration was determined on the SSP and sarcoplasmic fractions using a micro-Kjeldahl method 981.10 (AOAC, 1990). Non-protein nitrogen in the sarcoplasmic fraction was determined by precipitating protein nitrogen with 12.5% trichloroacetic acid (Kim et al., 1987). The amount of sarcoplasmic and myofibrillar protein in the muscle was calculated from the weight of supernatant and percentage protein in low and high salt extraction supernatants, respectively. Salt soluble proteins were diluted to 4% (40 mg/ml) (w/w) with 0.6 M NaCl, 0.05 M sodium phosphate buffer at the desired final pH. Protein yield was calculated as weight of protein (sarcoplasmic and salt-soluble) per muscle weight extracted multiplied by 100.

3.3.3 HELANDER EXTRACTION

Proteins were also extracted by the method of Helander (1957) and extraction yield compared to the SSP extraction procedure described previously. Ground muscle (25 g) was blended in a Waring Blendor (Model 1120) for two intervals of 45 sec each with 10 volumes of 0.03 M potassium phosphate buffer, pH 7.4. This solution was stirred with a T-Line laboratory stirrer (Talboy Engineering Corp.) at low speed for 3 hr in a cooler (2°- 4°C) and then centrifuged for 20 min at 1,400 x g. The supernatant was saved and the muscle residue was re-extracted with the same buffer using the same conditions. The supernatants from both extractions, which contained the sarcoplasmic protein, were combined. Total nitrogen and non-protein nitrogen were determined as previously described. The amount of sarcoplasmic protein was calculated from supernatant weight and protein content of supernatant (after subtracting non-protein nitrogen).

Another 25 g portion of ground muscle was extracted with ten volumes of 1.1 M KI, 0.1 M potassium phosphate buffer, pH 7.4. The ground meat was blended in a Waring blendor for two intervals of 45 sec each. The solution was stirred for 3 hr and centrifuged at 1,400 x g for 20 min. The supernatant was saved and the residue was re-extracted using the same procedure. Supernatants were combined and the pellet was extracted under identical conditions for 2 hr. Total nitrogen content and non-protein nitrogen content of the supernatant were determined. The supernatant contained both sarcoplasmic and myofibrillar proteins. The amount of myofibrillar protein was calculated by subtracting the quantity of the sarcoplasmic proteins and the non-protein nitrogen from the total nitrogen in the supernatant. The amount of muscle

myofibrillar protein was determined using the weight of supernatant and protein content of the myofibrillar fraction. Protein yield was calculated as in section 3.3.2.

3.3.4 AGING STUDY

Prerigor semimembranosus muscle was used to determine the effect of aging on protein extractability. The muscle was divided into three portions (about 125 g) and analyzed at 0, 7, 14 days after slaughter. The 7 and 14 day samples were vacuum packaged and stored at 2° to 4°C. Sarcoplasmic and salt soluble proteins were extracted using the method in section 3.3.2 at 0, 7 and 14 days. Protein content by micro-Kjeldahl method and electrophoresis of SSP and sarcoplasmic protein fractions were done at each sampling time.

3.3.5 PROXIMATE ANALYSES AND pH

Moisture, fat, and protein content of the ground muscle were analyzed following AOAC methods (1990), 950.46 B, 991.36, 981.10, respectively. Determinations were performed in triplicate for each extraction.

For pH determination, 10 g meat were homogenized with 50 g deionized water using a Polytron homogenizer (Model PT 10/35, Brinkmann Instrument, Inc. Westbury, NJ 11590) equipped with a PTA 10TS generator. The sample was stirred for two periods of 30 sec at 4.5 speed setting and pH was measured while the solution was continuously mixed over a magnetic stirrer. The pH was determined in triplicate for each muscle.

3.3.6 PROTEIN SOLUBILITY

Solubility was determined following Morr et al. (1985) with modifications in the procedure to adjust the solution pH. Thirty grams of SSP from each muscle (40 mg/mL) were weighed into a 150 mL beaker and the pH was adjusted in 0.5 pH units from 6.0 to 7.5 and in 0.2 pH units from 5.0 to 6.0. SSP was diluted with 3 volumes of 0.6 M NaCl, 0.05 M sodium phosphate buffer at the desired final pH to obtain a final concentration approximately 10 mg/mL. If necessary, pH was adjusted with 0.1 M NaOH or 0.1 M HCl. The solution was transferred into 50 mL centrifuge tube and shaken overnight in a Dubnoff (Precision Scientific, Chicago, IL 60647) shaking incubator in a cold room at 2°- 4°C at speed setting of 2.

The next day, samples were centrifuged at 20,000 x g for 30 min at 2°C. The protein concentration of the solution before centrifugation and supernatants (designated as soluble protein fractions) were determined by biuret method (Cooper, 1977). Percent solubility was determined by dividing the supernatant protein concentration by the solution concentration and multiplying by 100.

3.3.7 GEL PREPARATION

Four percent (40 mg/mL) SSP (prepared as described in section 3.3.2) was used to prepare gels as described by Beuschel et al. (1992). Air bubbles were removed using a vacuum mixer (Model UMC 5, Stephan Universal Machine, Columbus, OH 43228) by applying a vacuum (101.3 kPa) for five 1-min periods. SSP solutions were mixed at 300 rpm for 30 sec between each vacuum step. Temperature was monitored during vacuum mixing and kept under 18°C using a circulating bath. A 60 mL plastic

syringe (Becton Dickinson & Co., Rutherford, NJ 07035) was used to carefully pipette the SSP into glass tubes (10 x 130 mm) stoppered at one end. Tubes were covered with plastic caps. Gels were heated in a water bath at 73°C (Model MW-1120A-1, Blue M, Blue Island, IL 60406) to an internal temperature of 70°C, held at this temperature for 10 min, and cooled until the gel reached 10°C using ice water. The temperature was monitored using a thermocouple in a tube containing 4% SSP. The gels were stored overnight at 2° to 4°C. Gels were evaluated the following day.

3.3.8 GEL EVALUATION

3.3.8.1 SYNERESIS

Before texture profile analysis, each gel was weighed to measure the amount of free released water or syneresis from each sample tube. Syneresis was calculated as weight of water divided by total weight (gel plus water released) multiplied by 100.

3.3.8.2 EXPRESSIBLE MOISTURE

Expressible moisture was measured using the method developed by Kocher and Foegeding (1993) with some modifications. Salt soluble protein gels in 0.6 M NaCl, 0.05 M sodium phosphate at pH 6.05 were used to standardize centrifugation time. A 10 mm core was cut from a SSP gel. This core was cut lengthwise using a coffee straw (3.0 mm I.D.) giving a final gel of 10 mm x 3 mm. Gels were positioned in the inner portion of micro-centrifuge tubes, without filter (Whatman Centrifugal Filters, Series 7000, Model 6610N7168, Hillsboro, Oregon 97123). Gels were centrifuged at 365 x g for various times (2.5, 5, 7.5, 10, 20, 30,

60, and 90 min) and the amount of water expressed (by weight) was recorded. Data from time vs. expressed moisture showed that the time to obtain a constant value was 60 min. Expressible moisture was calculated as the weight of water released divided by gel weight multiplied by 100. Expressible moisture was determined four times in each of three replicates. Total water loss was calculated by adding the average of the syneresis and expressible moisture percentages and expressed as total percentage of water loss.

3.3.8.3 TEXTURE PROFILE ANALYSIS

Textural characteristics of gels were measured as described by Bourne (1978) using an Instron Universal Testing Machine (Model 4202, Canton, MA 02021) with a 50 N compression load cell at 25°C. Three to four cylindrical gel cores of 10 x 10 mm were cut with a blade from each of three tubes in each replicate. Gels located within 1 cm from the bottom and top of each tube were not used. There were three tubes for each of three replicates. Gel cores were uniaxially compressed to 80% of original height between two lubricated plates in two cycles at a crosshead speed of 30 mm/min. Apparent stress and strain at failure (Hamann, 1983), brittleness, hardness and springiness (Bourne, 1978) were calculated from the force-time curve.

Equations for calculating strain (ϵ), apparent strain (ϵ_{app}), and apparent stress (σ_{app}, KPa) were reported by Hamann (1983) and are described in equations 1, 2 and 3.

Strain (ϵ) was calculated as:

$$\epsilon = 1-L/L_0 \qquad (1)$$

where L = length of core at failure (cm)

L_0 = original length (cm)

Apparent strain (ϵ_{app}) was calculated as:

$$\epsilon_{app} = - \ln (\epsilon) \quad (2)$$

Apparent stress (σ_{app}, Pa) was calculated as:

$$\sigma_{app} = F / \pi r^2 (1 + \underline{\nu} \epsilon)^2 (1000) \quad (3)$$

where F = Force at failure from chart (N)

π = 3.14159

r = radius of core (m)

$\underline{\nu}$ = Poisson's ratio (0.48)

ϵ = Strain (dimensionless)

Brittleness was the force (N) at the initial fracture point of the gel. Hardness was calculated as the peak force (N) during the first compression cycle. Springiness was the height (mm) that the gel core recovered between the first and second compression.

3.3.8.4 TORSION

The stress and strain at failure of the protein gels was measured by the torsion technique (Hamann, 1983). The torsion gelometer system (Gel Consultants, Inc., Raleigh, NC 27612) used included a milling machine (Model 91) and a viscometer (Model DV1, Brookfield Engineering Laboratories, Inc., Stoughton, MA 02072). Sample cores (29 mm x 19 mm) were cut and the ends fixed to plastic disks using cyanoacrylate adhesive (Wonder Bond Plus, Borden, Inc., Columbus, OH 43215). Two sample cores per tube and two tubes in each of two replicates were used. The gels were then milled into a capstan shape (with a center diameter of 10 mm) using the milling machine (Model 91) and analyzed in the

viscometer at a speed of 2.5 rpm at 25°C. The following equations were used to calculate stress and strain at failure (Diehl et al., 1979):

$$\tau = \text{viscosity reading} \times 1.581$$

where: τ = stress at failure (Pa)

1.581 = stress equation constant

viscosity reading = reading on viscometer at failure

$$\gamma = (0.150 \times t) - (0.00818 \times \text{viscosity reading})$$

where: γ = Strain at failure

0.150 = uncorrected shear strain constant

t = time it takes for sample to fracture (sec)

0.00818 = corrected stain equation constant

viscosity reading = reading on viscometer at failure

$$TSS = \ln(1 + (\{\gamma^2\}/2) + \gamma(1 + (\gamma^2/4))^{0.5}$$

where: TSS = True shear strain

γ = strain at failure

3.3.9 DYNAMIC TESTING

A Rheometrics Fluid Spectrometer (RFS-8400, Rheometrics, Inc., Piscataway, NJ 08854) equipped with a 50 mm diameter parallel plate device was used to perform oscillatory dynamic testing of 4% SSP solutions while heating from 25°C to 85°C at 1°C/min rate. The transducer was 100 g-cm. The sample cup was heated with a silicone oil circulating system and temperature was controlled by a temperature programmer (MTP-6 Micro-processor, Neslab Instruments, Inc., Newington, NH 03801). Three or four milliliters of SSP solution were loaded in the sample cup and a gap of 1-1.5 mm was set between the upper and lower plates. Optimum instrumental parameters, where strain was independent

of frequency (linear range), were established by conducting strain (0.01%-100%) and frequency (0.1-10 rad/s) sweeps at 25°C and 85°C.

Storage modulus (G') and loss modulus (G") were recorded every 0.5 min at a fixed frequency of 1 rad/sec and 3% strain during the controlled heating process. The heating program was as follows: hold for 3 min at 25°C, heat at 1°C/min to 85°C and final hold at 85°C for 2 min. Initial and final storage modulus and loss modulus and the temperature when G' reached 10 Pa for each rheological curve were recorded.

3.3.10 ELECTROPHORESIS

A Mini-Protean II dual slab cell (Bio-Rad Laboratories, Richmond, CA 94804) was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a Tris(hydroxymethyl)aminomethane glycine electrode buffer system (0.025 M Tris pH 8.3, 0.192 M glycine, and 0.1% sodium dodecyl sulfate (SDS)) as described by Laemmli (1970). The acrylamide stock solution was prepared by dissolving 30 g of acrylamide and 0.8 g N'-N methylene bisacrylamide in 100 mL of double distilled H_2O. The resolving gel contained 12% acrylamide in a TrisHCl-SDS solution (0.375 M TrisHCl buffer, 0.1% SDS, pH 8.8) and the stacking gel contained 4% acrylamide in a TrisHCl-SDS solution (0.125 M TrisHCl buffer, 0.1% SDS, pH 6.8).

Salt soluble proteins from SM and VI muscles were diluted to 2 mg/mL and sarcoplasmic proteins were diluted to 1 mg/mL using sample buffer (0.0625 M Tris, pH 6.8, 2% SDS, 10% Glycerol, 5% ß-mercaptoethanol, and 0.2% bromophenol blue), and mixed with a vortex mixer (Vortex-genie, Fisher Scientific, Pittsburgh, PA 15238) at speed 3

for 2 min. Then, samples were heated in boiling water for 5 min and stored at -20°C until used. Molecular weight standards (12μg, SDS-6H, Sigma Chemical Co., St Louis, MO 63178) or 10 μg protein solution were loaded into the sample wells using a 10 μl Hamilton syringe. A constant voltage of 200 volts (with 45 mA current setting) were applied using a Bio-Rad power supply (Model 1000/500, Richmond, CA 94804) until the tracking dye reached the bottom of the resolving gel in about 50 min. Gels were stained for 30 min with 0.25% Coomassie Brilliant Blue R250 in acetic acid-methanol-water (9:45:45, v/v/v). Several changes of an acetic acid-methanol-water solution (6:4:7) were used for destaining the gels (usually 1 to 3 hr) until the gel background was clear. The gel was preserved in 7.5% acetic acid solution.

The relative mobilities of the SSP proteins were used to estimate their molecular weights by comparing to relative mobilities of the molecular weight standards under the same electrophoretic conditions (Weber and Osborn, 1969).

3.3.11 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A completely randomized design was used. A two way analysis of variance (ANOVA) was performed to test the significance between muscle, pH, and muscle and pH interaction uding F-test (MSTAT, 1993). Bonferroni's test was used to compare the means of the four combinations of muscles and pH when there was significant interaction brtween muscle and pH. Two replicates were analyzed for the torsion test; three replicates were done for pH solubility profile, compression, and texture profile analyses; and four replicates were used for extraction efficiency and dynamic testing. VI and SM were obtained from 10

different animals to have sufficient muscles for all analyses. Proximate analysis and pH were determined on each muscle giving 10 replicates for these analyses.

3.4 RESULTS AND DISCUSSION

3.4.1 Proximate composition

The proximate composition of VI and SM muscle are shown in Table 3.1. The fat content of VI was 5.0% as compared to 1.5% fat for SM ($p > 0.001$). These values were lower than that reported for choice grade four year old heifers by Ramsbottom and Strandine (1948) of 7.8% and 2.9% fat for VI and SM, respectively. Marchello et al. (1968) studied fat content in heifers. The triceps brachii (red muscle) fat content was 7.1% compared to semimembranosus at 5.4%, measured as an ether extract on a wet basis. In our study the heifers were younger and the muscles were trimmed as much as possible. However, fat percent of m. cutaneus trunci (white muscle, 0.85%) and m. masseter from Norwegian red cattle were lower compared to ours (Meyer and Egelandsdal, 1992). The VI muscle contained more moisture (ca. 1%) and less protein (ca. 4%) than SM ($p > 0.05$). These values followed the same trend for white and red muscle in the Meyer and Egelandsdal study (1992). Cassens and Cooper (1971) found that the lipid content of red muscle was higher than white muscle in pigs. Moreover, red muscles (trapezius) in pig had 2.5 times more fat than a white muscle (longissimus dorsi) (Beechel et al., 1965).

The pH of SM was 5.46 and was 0.68 units less than VI ($p > 0.001$). Breidenstein et al. (1968) reported a similar pH for SM (pH= 5.35) measured using a probe. VI and SM muscle pH reported by Ramsbottom and

Table 3.1 Proximate analysis (wet weight basis) and pH of *vastus intermedius* and *semimembranosus* muscles from ten 16 to 24 month old heifers[a]

Parameter	*vastus intermedius*	*semimembranosus*
Moisture (% w/w)	75.5 ± 0.9[b]	74.5 ± 1.3
Fat (% w/w)	5.0 ± 1.3[c]	1.5 ± 0.9
Protein (% w/w)	18.0 ± 0.5[b]	22.0 ± 0.5
pH (% w/w)	6.14 ± 0.11[c]	5.46 ± 0.03

[a] Each value is mean ± standard deviation.
[b] Values are significantly different at $p \leq 0.05$ between **VI** and **SM**.
c Values are significantly different at $p \leq 0.001$ between **VI** and **SM**.

Strandine (1948) were 5.6 and 5.4, respectively. There was a difference of 0.80 pH units between cutaneus trunci (white muscle) and masseter (red muscle) in Norwegian red cattle (Meyer and Egelandsdal, 1992). The difference in ultimate pH has been reported in chicken breast (pH 5.5) and thigh (pH 6.0-6.1) and turkey breast (pH 5.6) and thigh (pH 6.1) (Amato et al., 1989). Foegeding (1987) reported slightly different pH values for turkey breast and thigh (6.0 and 6.4, respectively). Lawrie et al. (1963) measured the pH of different muscles in pigs. The longissimus dorsi (white muscle) had a pH of 5.68, compared to extensor carpi radiali (red muscle) with a pH 6.42. A lower ultimate pH is characteristic of muscles with a higher percentage of white fiber type, due to more extensive anaerobic glycolysis after death with consequent accumulation of lactic acid (de Fremery and Pool, 1963).

3.4.2 Method efficiency

Two different extraction procedures were used to compare protein extractability from VI and SM (Table 3.2). In the Nuckles method (Nuckles et al., 1990), each muscle was subjected to two low salt extractions followed by two high salt extractions. Sarcoplasmic protein and salt soluble protein of VI and SM muscles did not differ significantly. When sarcoplasmic and salt soluble protein fractions were added and reported as total soluble protein, VI contained 0.6% lower soluble protein than SM muscle. The extractability difference between muscles was more apparent when protein yield was calculated as gram of protein extracted per gram of fresh muscle. SM yielded 0.16 g protein/g muscle compared to 0.13 g protein/g muscle for VI. Higher extractability of protein from white muscle types than red muscle types

Table 3.2 Protein extraction efficiency and extraction yield of two bovine muscles at pH 7.4[a]

Protein Fraction	vastus intermedius		semimembranosus	
	Nuckles[b]	Helander[c]	Nuckles	Helander
Sarcoplasmic (% w/w)	24.6 ± 2.5	20.8 ± 4.6	23.6 ± 2.1	19.4 ± 4.0
Salt soluble (% w/w)	47.5 ± 5.7	55.5 ± 5.4	49.0 ± 5.9	53.5 ± 5.4
Total soluble (% w/w)	72.0	76.2	72.6	72.9
Non-protein Nitrogen (% w/w)	9.1 ± 0.8	10.2 ± 1.8	10.4 ± 0.4	10.9 ± 0.3
Protein yield $\frac{\text{g protein}}{\text{g muscle}}$	0.13 ± 0.02	0.13 ± 0.01	0.16 ± 0.02	0.16 ± 0.02

[a] Each value is mean ± standard deviation of four determinations.
[b] Nuckles et al., 1990.
[c] Helander, 1957.

across species is commonly reported in the literature (Foegeding, 1987; Parsons and Knight, 1990; Xiong and Brekke, 1989). Differences in the Z-bands (Offer and Trinick, 1983), presence of different protein isoforms in the M-protein (Parsons and Knight, 1990), and level of proteolytic activity (Xiong and Brekke, 1991b) have been given as possible reasons for differences in extractability between red and white muscle types.

The Nuckles method extracted more of the sarcoplasmic or low ionic strength soluble proteins (3.8% higher for VI and 4.2% higher for SM) than the Helander method. The Helander method used only phosphate buffer for sarcoplasmic protein extraction, while the Nuckles method also used 0.1 M NaCl. Asghar et al. (1985) stated that some sarcoplasmic proteins are not soluble in water. A low salt solution is needed to completely extract the sarcoplasmic proteins. The Helander method probably did not extract those sarcoplasmic proteins that required a low salt concentration for solubilization.

Salt soluble protein was extracted 3 times in 1.1 M KI in the Helander method. The Helander method resulted in a higher percentage of salt soluble protein than the Nuckles method giving the higher ionic strength of extraction buffer and the use of KI instead of NaCl. For SM there was a 4.5% difference in salt soluble protein between both methods and for VI this difference was almost doubled. The percentage of total extractable protein for SM was similar in both methods. However, VI total extractable protein was 4% lower with the Nuckles method than the Helander method. Both extraction methods gave the same protein yield for each muscle.

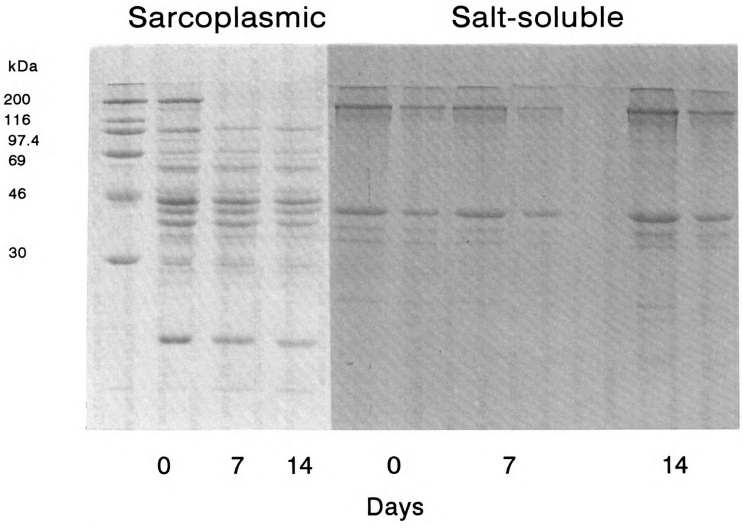
3.4.3 Aging Study

Ouali (1990) studied the effect of different proteolytic enzymes (cathepsins L and D and papain) on sarcoplasmic and myofibrillar protein during aging of beef muscles. In their study, fast-twitch muscles or fibers were more susceptible to proteolysis than slow-twitch muscles. For this reason, SM (white muscle) was used in our study to examine the effect of aging time on protein extraction. Figure 3.1 shows a sodium dodecyl sulfate-polyacrylamide gel of sarcoplasmic and salt-soluble protein extracted from SM muscle aged for 0, 7 and 14 days.

Sarcoplasmic protein composition was similar at 0, 7 and 14 days, except for the disappearance of a 205,000 dalton band and the decrease in a 95,000 dalton band on day 7 and 14. These bands were tentatively identified as myosin and α-actinin. Myosin and other salt soluble proteins can be extracted in small quantity by low salt or ionic strength buffer at pH 7 when the muscle are in pre-rigor state giving the presence of ATP . Richardson and Jones (1987) found that the amount of soluble proteins extracted from turkey varies with pH. The same pH dependency was reported for chicken by Xiong and Brekke (1991). This suggested that the combination of high extraction buffer pH (pH 7) and use of prerigor muscle might result in the extraction of some myosin with the low salt buffer. Xiong and Anglemier (1989) found that α-actinin was soluble on aging in low ionic strength buffer and coincided with the appearance of a 95,000 dalton band in the sarcoplasmic fraction.

There were no differences in salt soluble protein extracted at 0 and 7 days. Two bands of about 95,000 and 30,000 dalton appeared on day 14. The band around 95,000 dalton has been determined to be a

Figure 3.1. SDS-PAGE of sarcoplasmic and salt soluble proteins extracted from semimembranosus bovine muscle at different aging times (days) at 4°C.

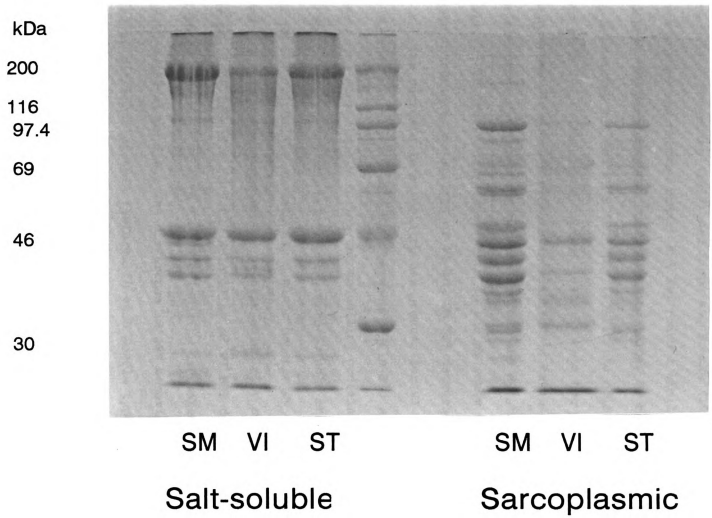

Sarcoplasmic
Salt-soluble
kDa
200
116
97.4
69
46
30
0
7
14
0
7
14
Days

proteolytic fragment that appears during aging, but its origin is unknown (Koohmaraie, 1994). Cheng and Parrish (1978) suggested the 95,000 dalton band might be α-actinin, because the weakening of Z-disks during aging of muscle could increase the extractability of α-actinin. The other band of 30,000 dalton was a proteolytic fragment of troponin T (Ho et al., 1993; Koohmaraie et al., 1984 a,b,c; Olson and Parrish, 1977). Myosin and actin were not affected during the aging period. This finding agreed with Koohmaraie (1994) who reported that myosin and actin were not affected during postmortem storage. These proteins are responsible for gelation and binding in processed meat. For this reason, in all subsequent experiments, muscles were obtained between 7 to 14 days after slaughter.

3.4.4 Electrophoresis of VI and SM protein fractions

The SDS-PAGE profiles of the sarcoplasmic protein fraction of each muscle type are shown in Figure 3.2. The sarcoplasmic proteins from **SM** (white muscle) were more numerous than those from **VI** (red muscle) or **ST** (intermediate type muscle type). The salt soluble proteins profile were similar; only the ratio of myosin and actin were different among muscle types. There were three bands in the region of troponin and tropomyosin for **VI** and only two bands for **SM**. Xiong and Brekke (1991) found two bands for chicken leg (red muscle) and only one for chicken breast (white muscle) in this region. The second and third bands in the **SM** and **VI** salt soluble protein could be the 30,000 dalton proteolytic fragment of troponin T that appears during aging. The myosin: actin ratio appeared visually to be higher in **SM** muscle than the other two muscle types.

Figure 3.2. SDS-PAGE of bovine salt soluble and sarcoplasmic proteins extracted from semimembranosus (SM-fast), vastus intermedius (VI-slow), and semitendinosus (ST-intermediate) muscles.


kDa
200
116
97.4
69
46
30
SM VI ST
SM VI ST
Salt-soluble
Sarcoplasmic

3.4.5 Influence of pH on protein solubility

The solubility profile of **VI** and **SM** SSP was dependent on pH (Figure 3.3). Foegeding (1987) and Xiong and Brekke (1991) working with SSP from turkey and chicken breast and leg found a similar pH dependency. In both studies, the solubility (≤20%) of both muscles was lowest between pH 5.0 and pH 5.50. The isoelectric point (pI) of SSP is between pH 5.0-5.4 (Xiong and Brekke, 1990a). When the pH of a solution approaches the pI, protein-protein interaction is maximal and there is less electrostatic interaction between protein and H_2O, thus reducing solubility. In preliminary experiments in the pH range of 5.0 to 7.5 at 0.5 unit increments, solubility of **VI** and **SM** SSP decreased between pH 6.0-5.5. The decrease in solubility was greater for **VI** than **SM**.

Further experiments were done between pH 6.0-5.0 at 0.2 unit increments to more closely follow the solubility profile. **VI** SSP was 96.5% soluble at pH 7.5 and was slightly more soluble than **SM** (92.7%). **VI** and **SM** SSP solubility showed only a minor dependency on pH between 7.5 and 5.8. In these experiments, protein solubility decreased by 70% in the pH range from 6.0 to 5.0. Solubility of **VI** SSP decreased from 70.6% at pH 5.8 to 39.7% at 5.6. **SM** solubility decreased from 55.2% at pH 5.5 to 24.3% at pH 5.4. The difference in SSP behavior could be related to different protein isoforms in each muscle type. Below pH 5.4 the solubility of **VI** and **SM** SSP was very low. This was expected near the pI where there is more protein-protein attraction and less interaction between solvent and protein.

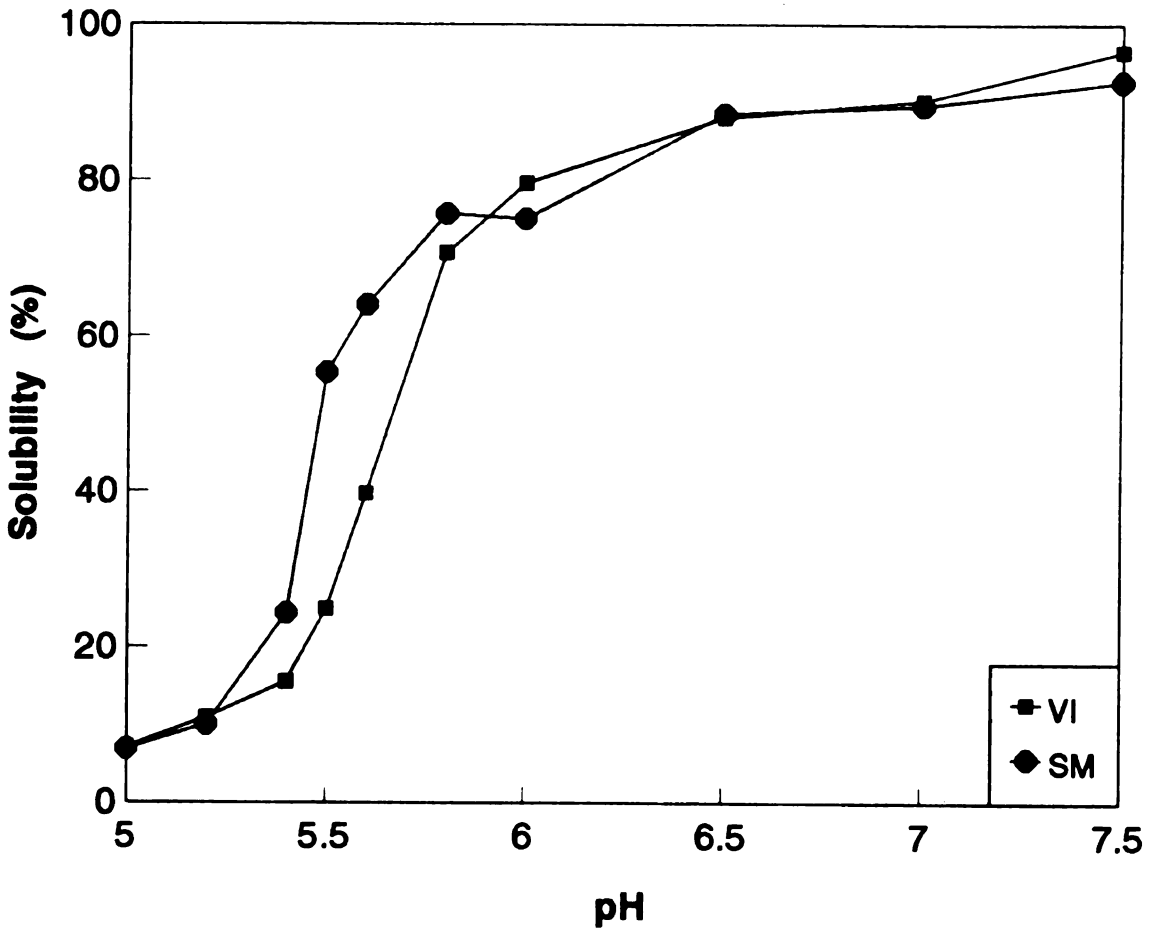


Figure 3.3. Solubility profile of semimembranosus (SM-fast muscle type) and vastus intermedius (VI-slow) salt soluble proteins in 0.6 M NaCl and 0.05 M sodium phosphate buffer as influenced by pH.

3.4.6 Gel Evaluation

3.4.6.1 Water retention properties

The effect of centrifugation time at 365 x g on expressible moisture of 4% SM SSP gels is shown in Table 3.3. There was no difference in expressible moisture after 60 min of centrifugation. Consequently, a centrifugation time of 60 min was used in all subsequent analyses as expressible moisture was constant and the standard deviation among measurements was low.

Gels prepared from VI had poor water holding ability (Table 3.4). This was shown by the increase in protein in the gel after heating and the higher percentage of gel syneresis. A higher percentage of syneresis corresponded to a higher final protein concentration in the gel due to loss of water. The initial protein concentration of SSP solutions was 4.0 ± 0.1%; after heating VI increased 1.4-1.6% compared with 0.9% for SM. The isoelectric point of SSP is close to pH 5.4-5.0. In general, protein has lower water holding ability at pHs near the isoelectric point. In our study this relation was sustained, given that gels from each muscle had higher syneresis at pH 5.50. However, syneresis of VI gels increased by 2% when the pH was adjusted to 5.50; in the case of SM, only a 0.5% increase in syneresis was recorded between pH 6.05 and 5.50.

Given the differences in final protein content of each gel, it was not possible to compare total water loss (syneresis and expressed moisture) among the four treatments. Another way to compare differences between muscles is to calculate a ratio of percentage expressible moisture to protein content of gels. The ratio of expressed moisture:protein for VI was lower at both pH's. This trend was reversed

Table 3.3 Effect of centrifugation time on the expressible moisture of 4% (w/w) salt soluble protein semimembranosus gels centrifuged at 365 x g

Time (min)	Expressible Moisture (%)[a,b]
2.5	42.8 ± 7.3
5.0	51.0 ± 4.4
7.5	57.9 ± 0.3
10.0	55.1 ± 4.0
20.0	66.1 ± 1.6
30.0	65.5 ± 3.3
40.0	68.0 ± 2.6
50.0	67.4 ± 3.6
60.0	67.3 ± 1.5
90.0	67.0 ± 1.2

[a] Weight of released water divided by original gel weight x 100.
[b] Each value is mean ± standard deviation of four determinations.

Table 3.4 Influence of muscle type and pH on expressible moisture and syneresis of beef muscle salt soluble protein gels[a]

	vastus intermedius		semimembranosus	
	pH 6.05	pH 5.45	pH 5.45	pH 6.05
Protein concentration of gel after heating (% w/w)	5.4 ± 0.4	5.6 ± 0.2	4.9 ± 0.1	4.8 ± 0.1
Syneresis (% w/w)	14.5 ± 1.7	16.5 ± 2.5	5.0 ± 2.1	4.7 ± 1.9
Expressible Moisture (% w/w)	67.1 ± 4.1	67.3 ± 1.3	69.3 ± 4.6	66.5 ± 1.7
Total water loss (% w/w)[b]	81.5	83.8	74.3	71.2

[a] Each value is mean ± standard deviation of four samples from each of three replications.

[b] Total water calculated by adding the mean of syneresis and expressible moisture percent.

when the whole ground cutaneus trunci (fast) was compared with masseter (slow) in 2.5% NaCl and heated at 70°C (Meyer and Egelandsdal, 1992). Hamm (1960) reported that the water holding capacity of longissimus dorsi (white muscle) from cattle was about twice that of psoas (red muscle) at the same pH. In turkey and chicken, leg SSP gels lose more water than breast SSP (Xiong and Brekke, 1990b). Differences in the gel microstructure are related to variations in water retention (Hermansson, 1982). Xiong and Brekke (1990b) postulated that protein isoforms from different skeletal muscle types could produce different gel matrices. Expressible moisture of 4% SSP gels from both muscles was similar at each pH. However, both muscles had a higher expressible moisture at pH 5.50.

3.4.6.2 Failure, Texture Profile and Torsion Analyses

When measured by compression, the apparent stress at failure of SSP gels from each muscle was different ($p \leq 0.12$) (Table 3.5). SSP from SM (white muscle) formed a firmer gel than VI at both pHs. These results agreed with Young et al. (1992) who found that cutaneus trunci (fast) gels were stronger than masseter (slow) gels at the same pH. Foegeding (1987) found that turkey breast SSP formed gels at a lower concentration than turkey thigh, and at the same concentration breast gels were stronger. Apparent strain of SSP gels did not differ with muscle type or pH.

Stress and strain at failure measured by the torsion test showed interactions between muscle type and pH. For this reason, the Bonferroni's test was used to compare means between muscles at each pH, and both muscles at the same pH. The pH had no effect on stress at

Table 3.5 Compression[a] and torsion[b] failure analyses of 4% (w/w) salt soluble protein gels in 0.6 M NaCl and 0.05 M sodium phosphate buffer

Muscle	Compression		Torsional	
	Stress (kPa)	Strain (dimensionless)	Stress (kPa)	Strain (dimensionless)
vastus intermedius				
pH 6.05	12.8 ± 2.0	1.0 ± 0.1	4.0 ± 1.0	1.8 ± 0.1
pH 5.50	13.9 ± 0.3	1.0 ± 0.0	6.3 ± 1.0	1.5 ± 0.2
semimembranosus				
pH 5.50	14.7 ± 1.5	0.9 ± 0.1	5.0 ± 0.6	1.6 ± 0.1
pH 6.05	14.5 ± 0.6	1.0 ± 0.1	5.2 ± 0.8	2.0 ± 0.1

[a] Each value is mean ± standard deviation of three replications of three tubes with three samples per tube.

[b] Each value is a mean ± standard deviation of four replications of four tubes with three samples per tube.

fracture of SM SSP gels. The pH had a significant effect ($p \leq 0.05$) on stress at fracture of VI SSP gels. VI gels prepared at 5.50 were firmer than gels at 6.05. Strain at fracture did not differ between muscles at pH 6.05 and gave higher values than at pH 5.50. The higher strain at pH 6.05 indicated a more elastic gel at this pH. Hamann (1983) suggested that the degree of elasticity is related to the number of crosslinks in the protein gels.

When evaluated by texture profile analysis, brittleness did not show an interaction, whereas pH had a significant effect in each muscle at $p = 0.25$ (Table 3.6). Both VI and SM gels had the lowest brittleness at their own ultimate pH. SM gels showed higher value for hardness and springiness than VI. Gel hardness and springiness showed interaction between variables. No significant differences in gel hardness or springiness were found due to pH or muscle type.

3.4.6.3 Viscoelastic properties

The storage modulus, loss modulus, and tan δ of each muscle during heating from 25 to 80°C at 1°C/min is shown in Figure 3.4, 3.5 and 3.6. The general behavior of each muscle at its ultimate pH will be described and then differences at the other pH will be pointed out (Table 3.7).

For VI at pH 6.05, the storage modulus (G'= elastic behavior) was constant until 55-56°C, then increased to a peak at 62.3°C. After reaching this peak, G' decreased to about 65°C, then increased again to 72°C. No changes in G' were observed between 72°C and 80°C. Loss modulus (G"= viscous behavior) increased to a peak at 62°C, then decreased during the rest of heating period. However, the magnitude of change in G" was lower than storage moduli. Tan δ (G"/G'= relative

Table 3.6 Texture profile parameters of 4% (w/w) salt soluble protein gels in 0.6 M NaCl and 0.05 M sodium phosphate buffer heated to 70°C[a]

Muscle	Brittleness (N)	Hardness (N)	Springiness (mm)
<u>vastus intermedius</u>			
pH 6.05	1.7 ± 0.2[b]	2.2 ± 0.3	0.4 ± 0.1
pH 5.50	1.9 ± 0.1[c]	2.4 ± 0.1	0.5 ± 0.0
<u>semimembranosus</u>			
pH 5.50	1.9 ± 0.2[b]	2.8 ± 0.3	0.5 ± 0.0
pH 6.05	2.0 ± 0.1[c]	2.7 ± 0.1	0.5 ± 0.0

[a] Each value is mean ± standard deviation of three replications of three tubes with two samples from each tube.
[b,c] Values were significantly different at $p \leq 0.25$.

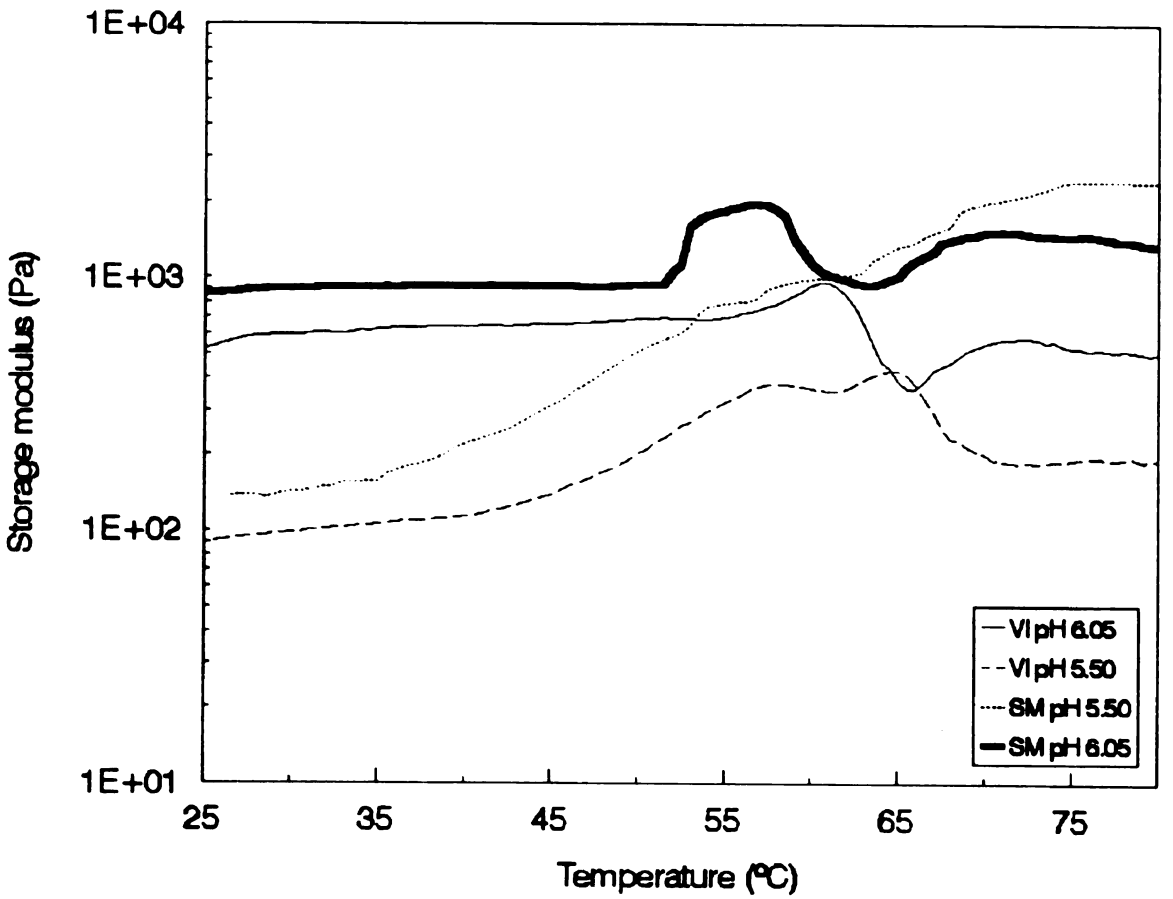


Figure 3.4. Storage modulus (G') of 40 mg/mL salt soluble proteins from semimembranosus (SM - fast) and vastus intermedius (VI - slow) muscles heated at 1°C/min in 0.6 M NaCl and 0.05 M sodium phosphate buffer at pH 5.50 and 6.05.

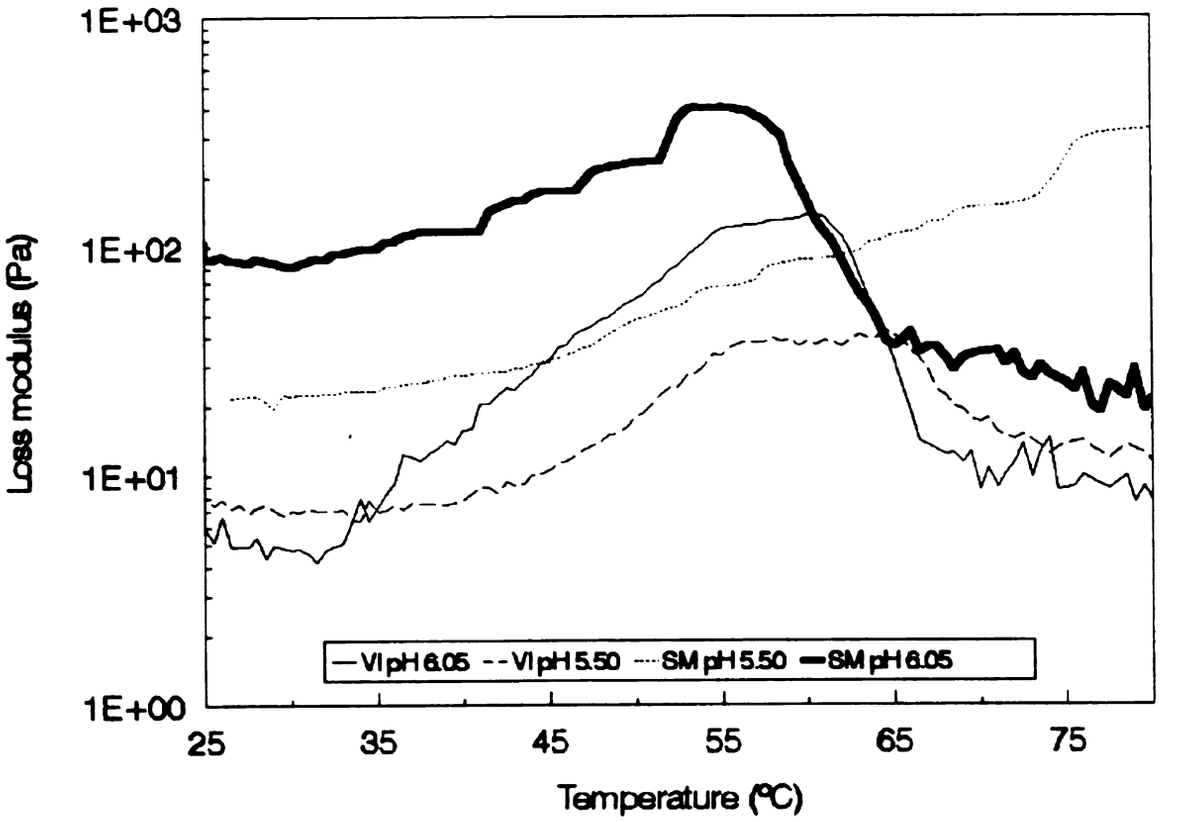


Figure 3.5. Loss modulus (G") of 40 mg/mL salt soluble proteins from semimembranosus (SM - fast) and vastus intermedius (VI - slow) muscles heated at 1°C/min in 0.6 M NaCl and 0.05 M sodium phosphate buffer at pH 5.50 and 6.05.

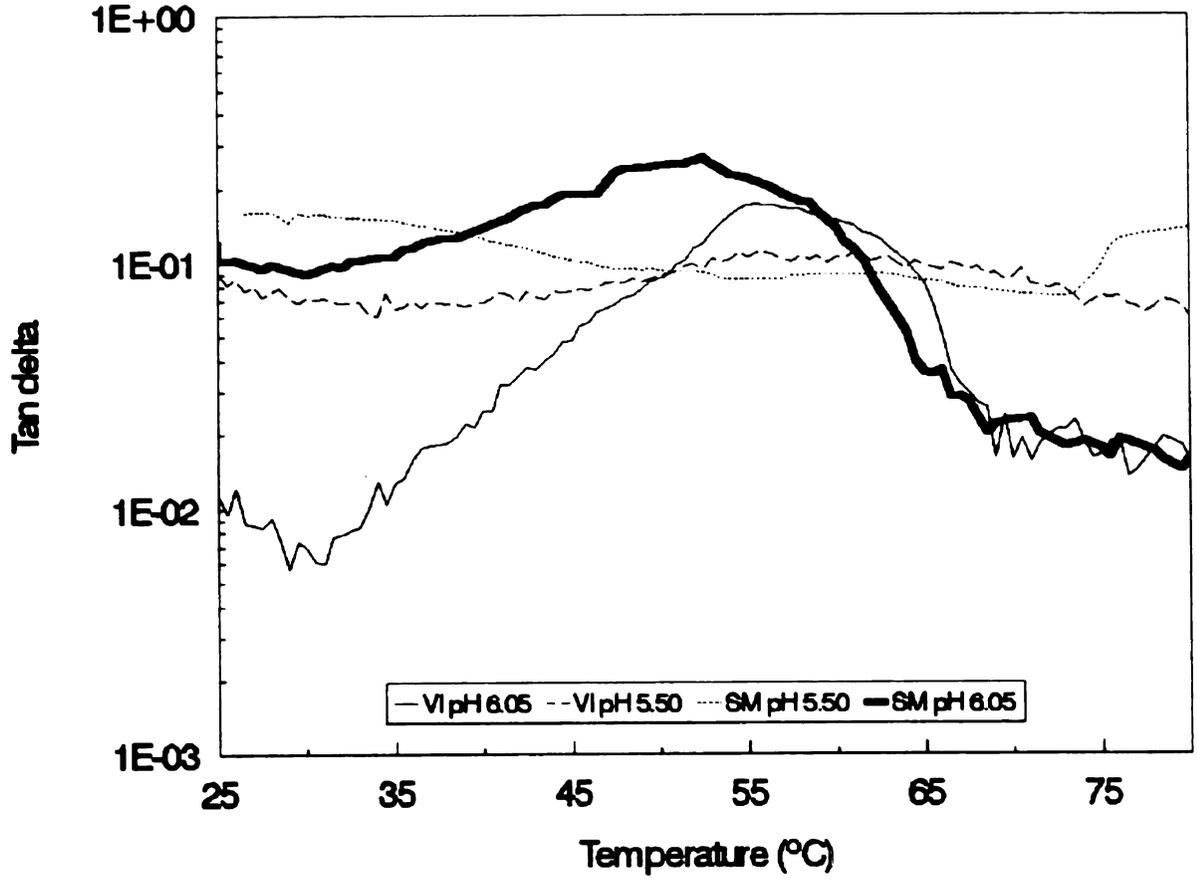


Figure 3.6. Tan delta (tan δ) of 40 mg/mL salt soluble proteins from semimembranosus (SM - fast) and vastus intermedius (VI - slow) muscles heated at 1°C/min in 0.6 M NaCl and 0.05 M sodium phosphate buffer at pH 5.50 and 6.05.

Table 3.7 Dynamic rheological properties and transition temperatures of 4% (w/w) salt soluble protein solutions in 0.6 M NaCl and 0.05 M sodium phosphate buffer heated from 25 to 80°C at 1°C/min[a]

Parameters	vastus intermedius		semimembranosus	
	pH 6.05	pH 5.50	pH 5.50	pH 6.05
Storage moduli (G' Pa)				
initial	470.7	237.3	172.0	760.0
peak	1160.7	823.8	1076.2	2075.5
final	1224.2	268.6	1868.7	1162.8
Loss moduli (G" Pa)				
initial	32.3	41.0	44.5	85.4
peak	123.5	198.4	82.1	311.5
final	33.2	91.5	44.8	118.2
Temperature (°C)				
G' peak	62.3	59.0	52.7	56.3
G" peak	61.9	63.7	55.0	54.7
Tan δ				
initial	0.08	0.30	0.27	0.12
final	0.02	0.28	0.06	0.04

[a] Each value is mean of three replications.
[b] initial is the parameter value at 25OC, peak maximmun value and final is the value at 80OC

viscous:elastic behavior) decreased after 50°C during heating indicating the formation of a gel matrix.

When the pH of VI was adjusted to 5.50 (ultimate pH of white muscle), the storage moduli during heating was different than that at 6.05. The initial G' (25°C) was less than VI at pH 6.05. The storage moduli started to increase at 45°C and the rate was higher than VI at pH 6.05. Two transition peaks were observed at pH 5.50; one peak at 51-54°C and a second around 65-67°C. The G' then decreased from 67°C to 80°C. Loss modulus followed the same pattern as G'. However, final G" was higher at pH 5.50 than at pH 6.05. At 80°C, tan δ of VI gels at pH 6.05 was lower than VI at pH 5.50 indicating a more elastic gel matrix. The initial and final values of tan δ were very close at pH 5.50.

For SM (white muscle) at pH 5.50, G' followed a different pattern than the other treatments during heating. The storage modulus increased steadily with increasing temperature. The initial G' (25°C) was 172 Pa and increased to 75°C. The G' at 80°C of SM gels at pH 5.50 was the highest of the four treatments. In this case, the G' profile showed the same slope as VI at pH 5.5 up to 55°C. Two small peaks were observed at 57 and 62°C. The G" was constant to 42-44°C, increasing steadily to 75°C. Tan δ changes were very small during the entire heating period.

At pH 6.05 (ultimate pH of red muscle), G' of SM was constant until 51-53°C, then increased very rapidly to a broad peak 55-57°C. The peak G' was 2075 Pa, the highest of the four treatments, G' decreased until 64°C, then increased very slowly up to 80°C. The G" was constant to 42°C, followed by an increase to a peak at 55°C, then there was a steady decrease during the rest of the heating period. The pattern of tan δ was very similar to G" profile.

VI and **SM** gel profiles during heating were similar at pH 6.05. Both muscles showed a peak in G' followed by a decrease, then G' slowly increased to 75°C. The major differences between **VI** and **SM** were the temperatures at which these changes in G' occurred and the magnitude of G' values. In this case, **SM** (the white muscle) had higher G' throughout heating and the transition temperature range was broader. Moreover, the temperature at which the G' peak occurred for **SM** was between 6 to 7°C lower than the temperature for **VI**. Young et al. (1992) reported a 10°C difference for bovine cutaneus trunci (white) and masseter (red) under slightly different conditions using a thermal scanning rheometer. Meyer and Egelandsdal (1992) reported a higher complex modulus (G*) for whole ground cutaneus trunci (fast) than masseter (slow) with 2.5% NaCl. When pH was changed to 5.50, both muscles showed lower peak values, but the value of G' at 80°C was higher at pH 5.50. These results are similar to Xiong and Blanchard (1994b) with chicken breast and thigh myofibrillar proteins during heating from 20 - 70°C.

3.5 CONCLUSIONS

VI and **SM** muscles were very different in composition and ultimate pH. **VI** had a higher percentage of fat and a lower percentage of protein. The ultimate pH of **SM** was lower than **VI**. For both **VI** and **SM** muscles, the electrophoresis profiles of the sarcoplasmic and salt soluble proteins were similar. However, the extractability of SSP from **SM** was higher than for **VI**.

In the present study, the differences in gelation properties of salt soluble proteins were influenced by muscle type and ultimate pH of each muscle. The effect of pH on gel properties can be summarized as

follows: water retention in the gel matrix was lower and the onset temperature for gelation was at a lower temperature for SSP gel at pH 5.50 than gels at pH 6.05. The SSP gel at pH 6.05 had more viscous behavior than the gel at pH 5.50 as indicated by G' and tan delta at 80°C.

The effect of muscle type on gel properties can be summarized as: **SM** had at higher water retention properties and a lower onset gelation temperature. The **VI** SSP gel at pH 6.05 was more viscous than **SM**. However, this behavior was reversed at pH 5.50. This behavior suggested that each muscle type at its own ultimate pH had better functional properties. Meat processors can use this type of information to adjust formulations to maximize yield and product quality.

CHAPTER 4

THERMAL GELATION OF MYOSIN FROM TWO BOVINE MUSCLE TYPES

4.1 ABSTRACT

Myosin plays a major role in the binding and texture of processed beef products; however, gelation properties of myosin isoforms from different bovine muscle fiber types have not been thoroughly investigated. This study compared the thermal behavior of myosin from two bovine muscles: vastus intermedius (VI, predominantly red muscle) and semimembranosus (SM, predominantly white muscle). Pre-rigor muscle was ground, extracted in 5 volumes 0.266 M K_2HPO_4, 10 mM $Na_4P_2O_7$, 0.1 mM PMSF, followed by precipitation at low ionic strength and NH_4SO_4 fractionation. Myosin was dialyzed with 0.6 M NaCl, Na phosphate buffer at either pH 6.05 (ultimate pH of VI muscle) or 5.5 (ultimate pH of SM muscle) and purified by centrifugation at 78,000 x g. Protein unfolding, aggregation and gel formation was monitored by heating myosin solutions from 30 to 80°C at 1°C/min using differential scanning microcalorimetry (DSC), turbidity at 340 nm and dynamic rheological testing, respectively. VI and SM myosin heavy and light chains presented different mobilities in a gradient SDS-PAGE containing a glycerol gradient. Endotherms of VI myosin at pH 6.05 had three peaks with transition temperatures (Tm) of 45, 53, and 57°C, whereas at pH 5.60 it had two Tm of 42 and 59°C. SM myosin at pH 6.05 and 5.60 had two major Tm at 46°/58°C and 43°C/62°C, respectively. In dynamic

rheological testing the onset temperature to obtain a storage modulus (G') equal to 10 Pa and the final G' were measured. For **SM** myosin at its ultimate pH the onset temperature was 38°C compared with 55°C for **VI** myosin at pH 6.05; however, the final values of G' were similar. **SM** myosin at its own ultimate pH was less heat stable than **VI** myosin at its ultimate pH; however, when **SM** and **VI**' myosins are compared at each pH, **VI** myosin was less stable.

4.2 INTRODUCTION

Myosin is the most important protein in the gelation of processed meat. The myosin molecule consists of six separate polypeptides with a total molecular weight of about 500 kDa. The two large polypeptides are called myosin heavy chains and four smaller ones are called light chains.

Several different isoforms of myosin are found among the fiber types. Young (1982) showed, with peptide maps, that there are differences in myosin heavy chains from fiber types I and II. In slow myosin, only one heavy chain polypeptide was found per species (Billeter et al., 1981; Salviati et al., 1982; Young and Davey, 1981). Two different maps of myosin heavy chain were obtained from purified single bovine fast fibers which were different from the slow heavy chain and from each other (Young and Davey, 1981). Later, Young (1982) established that each variant was predominant in each of the two type II fiber types. These two peptide chains come from two different genes, but their molecular weight is approximately the same. The type II forms contain 2 residues of 3-methylhistidine, a unique amino acid, whereas red has none.

Myosin light chains vary with fiber type and have slightly different molecular weights. In the type IIb or fast-twitch three different myosin light chains have been identified: LC1f, LC2f and LC3f. The slow or red fiber contains two types of light chains LC1s and LC2s which are similar to LC1f and LC2f. During electrophoresis, light chains from slow muscle migrate more slowly suggesting a higher molecular weight relative to that of the light chains of white muscle.

There are extensive data in the literature regarding the gelling properties of myofibrillar proteins. Myosin by itself has been shown to be more functional than any of the other myofibrillar proteins, even actomyosin (Macfarlane et al., 1977). In the hexameric myosin molecule, the myosin heavy chain, and in particular the myosin rod, demonstrated gel properties similar to that of the intact myosin (Asghar et al., 1985; Ishioroshi et al., 1981; Samejima et al., 1981). The overall contribution of myosin light chains to gel strength is very small (Samejima et al., 1984).

In recent years, comparative studies have determined differences in gelation between red and white muscles or fibers from different species. Among the different species, white and red muscles have shown different behaviors. Across species, white muscle protein forms the strongest gel. Most of these studies have been done using chicken, given that the breast muscles are predominant in fast type and the leg muscles are slow type. In a recent review, Xiong (1994) stated that myosin from chicken breast muscle forms a stronger gel than the myosin isolated from leg under the same conditions. Liu et al. (1995) demonstrated that chicken pectoralis major (fast type muscle) myosin aggregated at a lower temperature than iliotibialis and gastrocnemius

(slow type) myosins.

Classically, the study of fiber type influence on gelation in bovine species had been done using two muscles, cutaneus trunci (fast type) and masseter (slow type). Cutaneus trunci myosin showed a higher solubility at pH 5.5 than masseter in 0.6 M NaCl in phosphate buffer and their solubilities were similar at pH 6.0 (Fretheim et al., 1986). Dynamic rheological properties showed a stronger gel was formed by cutaneus trunci when compared to that of a masseter gel at a pH greater than pH 5.8 in 0.2 and 0.6 M NaCl phosphate buffer. In the case of 0.6 M NaCl and pH < 5.8, masseter myosin formed a stronger gel than cutaneus trunci. More recently, Egelandsdal et al. (1994) stated that cutaneus trunci myosin was less stable than masseter myosin, given that during calorimetric measurements, the first transition peak of cutaneus trunci occurred at a lower temperature. Moreover, the authors found that when pH was decreased the temperature of the first transition was also decreased. The storage modulus of these two muscle myosins decreased when pH decreased from pH 5.75 to 5.50. Wang et al. (1990) reported similar changes in G' of chicken breast salt soluble protein with pH.

Different thermogelation behavior of myosin from different fiber types was suggested as a cause for differences in filamentogenesis. Another possible cause of differences in gelation properties of muscle fiber types is the pH of meat. The pH of red and white muscles are different in different muscles and may be attributable to different metabolic by-products, such as lactic acid (Daum-Thunberg et al., 1992). Red muscle has a higher pH than that of white muscle (Angel and Weinberg, 1981). These two parameters, filamentogenesis and pH, are closely related. Hermansson et al. (1986) showed that formation of

these two types of gels were dependent on the pH of the solution. These gel structures were a coarse network at pH 6.0 formed by head-to-head aggregates during heating, and more filamentous type gels at pH 5.50 when myosin tends to assemble into filaments. The objective of this research is to understand the influence of myosin isoform and ultimate pH on myosin gelation in two bovine muscles.

4.3 MATERIALS AND METHODS

4.3.1 MYOSIN EXTRACTION

Myosin was extracted from prerigor semimembranosus and vastus intermedius muscles of heifers between 16-24 months of age three different times as described by Swartz et al. (1993) without the cooling step. Muscles were removed from the carcass within 30-45 min of death and excess fat and connective tissues were trimmed. Each muscle was ground twice through a Kitchen Aid Food Grinder (Model K5-A, Troy, OH 45374) using a 4 mm plate.

Each muscle was suspended in 5 volumes 0.266 M K_2HPO_4, 10 mM $Na_4P_2O_7$, 0.1 mM phenylmethylsulfonyl fluoride. In the case of the first extraction, this slurry was mixed for two periods of 45 sec each in a Waring blendor. The suspension was stirred for 30 min with a T-Line laboratory stirrer (Talboys Engineering Corp., Emerson, NJ 18801) containing a 5.1 cm paddle propeller with three blades connected to a rheostat (Powerstat, The Superior Electric Company, Bristol, CT 06010) to control speed. The slurry was centrifuged at 10,000 x g for 20 min and the supernatant was filtered through glass wool. The pellet containing actin was discarded or saved for actin purification. The supernatant was adjusted to pH 6.7-7.0 by adding H_3PO_4 with continuous

stirring. The solution was diluted with 9 volumes 1 mM EDTA (pH 7.0) and allowed to precipitate overnight at 4°C. The supernatant was decanted and the precipitate was centrifuged at 3,500 x g for 20 min.

The pellet was washed two times in 20 mM KH_2PO_4 and 1 mM EDTA (pH 7.0), stirred for 10 min and centrifuged at 3,500 x g for 20 min. After the second wash, the pellet was weighed and solubilized with 0.5 volumes of a solution containing 1 M KCl, 10 mM KH_2PO_4 (pH 7.0), 1 mM EDTA to obtain a final concentration of 0.5 M KCl. If the pellet required more KCl for solubilization, then a 0.5 M KCl solution was used. The final volume of the suspension was determined and an equal volume of 2.7 M $(NH_4)_2SO_4$, 0.5 M KCl, 10 mM KH_2PO_4 (pH 7.0), 1 mM EDTA was added slowly and stirred for 20 min at 4°C. The suspension was centrifuged at 8,000 x g for 20 min, the pellet was discarded and the supernatant filtered through glass wool. The filtrate volume was determined and solid ammonium sulfate (57 g/L) was added slowly with stirring ,to achieve ca. 41% $(NH_4)_2SO_4$ or 35% $(NH_4)_2SO_4$ and then stirred for an additional 20 min to precipitate VI and SM myosin, respectively. By eliminating the use of the Waring blendor both myosins were precipitated in 41% $(NH_4)_2SO_4$. The myosin pellet was collected by centrifugation (8,000 x g) for 20 min and stored in 10% glycerol at -20°C for future use.

Myosin was suspended in 0.6 M NaCl, 0.05 M sodium phosphate buffer (pH 6.05 or pH 5.50), 1 mM EDTA, and dialyzed against three changes of buffer before it was used. Myosin was then dialyzed two more times against the same buffer, but without EDTA, and then centrifuged (78,000 x g) for 1 hr (Beckman Ultracentrifuge, Model L7-65, Beckman Instruments, Inc., Palo Alto, CA 94304). The concentration of myosin in the supernatant was determined using an extinction coefficient of $E^{1\%}$ =

5.5 at 280 nm (Swenson and Ritchie, 1980).

4.3.2 ELECTROPHORESIS

To determine the purity of the myosin, a Mini-Protean II dual slab cell (Bio Rad Laboratories, Richmond, CA 94804) was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The system was run using a Tris(hydroxymethyl)aminomethane glycine electrode buffer (0.025 M Tris pH 8.3, 0.192 M glycine) and 0.1% sodium dodecyl sulphate (SDS) as described by Laemmli (1970). The resolving and stacking gels contained 10% and 4% acrylamide, respectively.

Myosin from the **SM** and **VI** muscles were diluted to 2 mg/mL using sample buffer (0.0625 M Tris, pH 6.8, 2% SDS, 10% glycerol, 5% ß-mercaptoethanol, and 0.2% bromophenol blue) and mixed well using a vortex mixer (Vortex-genie, Fisher Scientific, Pittsburgh, PA 15238) at speed 3 for 2 min. The samples were then heated in boiling water for 5 min to denature the proteins and then stored at -20°C until used.

Molecular weight standards (12 µg, SDS-6H, Sigma Chemical Co., St Louis, MO 63178), bovine skeletal muscle myosin (5 µg, M-6643, Sigma Chemical Co.), or extracted myosin (6 µg) in a volume less than 10 µL were loaded into the sample well using a 10 µL Hamilton syringe. The gels were run at constant voltage (200 volts) for about 50 min, then stained for 30 min with 0.25% Coomassie Brilliant Blue R250 in acetic acid-methanol-water (9:45:45, v/v/v) solution. Gels were destained with 3-4 changes of an acetic acid-methanol-water solution (6:4:7) and then preserved in 7.5% acetic acid solution and stored at room temperature. Mobility of the extracted myosin was compared to that of the myosin standard. The molecular weight of the protein bands were estimated by

their relative mobilities and compared to that of the standard molecular weights under the same electrophoretic conditions (Weber and Osborn, 1969).

4.3.3 ELECTROPHORESIS OF MYOSIN ISOFORMS

Gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to determine the myosin heavy chain isoforms present in each muscle. A 5-8% acrylamide gradient and 30-40% glycerol gradient were used as described by Sugiura and Murakami (1990). The stacking gel was 3.5% acrylamide with 35% glycerol.

Myosin from SM and VI muscles were diluted to 50 ng/mL using sample buffer (0.0625 M Tris, pH 6.8, 2% SDS, 30% glycerol, 5% ß-mercaptoethanol, and 0.2% bromophenol blue) and mixed well using a vortex mixer. Samples were incubated for 10 min at about 60°C to denature proteins and then stored at -20°C until used.

Bovine skeletal muscle myosin standard (M-6643, Sigma) and samples (extracted myosins) were loaded at 300, 500 and 600 ng per well. Gels were run at 50 mV for 5 hr, increased to 100 mV for 2 hr, and then increased to 150 mV until the tracking dye reached the bottom of the gel. The entire run took about 8 hr and the electrophoretic cells were surrounded with ice. Due to the length of the run, cold electrode buffer was added every 90 min to maintain the correct volume.

Gels were stained with Coomassie Brilliant Blue R250 in acetic acid-methanol-water (9:45:45, v\v\v) solution, then destained with 3-4 changes of an acetic acid-methanol-water solution (6:4:7) and preserved in 7.5% acetic acid. Duplicate gels were also stained using a Silver stain kit (161-0443, Bio-Rad Laboratories). Mobility of the myosin

heavy chain bands were compared with the myosin standard and literature reports of myosin isoform separation (Betto et al., 1986; Hoh et al., 1976; Sugiura and Murakami, 1990).

4.3.4 WESTERN BLOT ANALYSIS

Myosin isoforms were identified by Western blot analysis using a Mini Trans-Blot unit (Bio-Rad Laboratories). Myosin from **VI**, **SM**, and standard from SDS-PAGE gradient gels were transferred electrophoretically onto a nitrocellulose membrane (0.45 μm, Schleicher & Schuell, Keene, NH 03431) for 1.5 hr at 100 V using 25 mM Tris, 192 mM glycine, and 20% (v/v) methanol buffer (pH 8.3).

The membrane was washed twice with PBS-Tween (0.14 M NaCl in sodium phosphate buffer pH 7.2, and 0.02% v/v Tween-20) after transferring, then blocked with 10 mL (per membrane) of filtered 3% egg albumin (Sigma) in PBS for 30 min at room temperature, and rinsed with PBS-Tween. Membranes were incubated with three different antibodies: anti-myosin skeletal (Sigma M 7523, polyclonal), anti-myosin skeletal fast (Sigma M 4276), and anti-myosin skeletal slow (Amersham RPN.1168, Arlington Heights, IL 60005). Ten milliliters of the appropriate antibody diluted 1:10 or 1:20 in 3% egg albumin-PBS was used for each membrane during incubation at room temperature for 30 min.

PBS-Tween washing was used to remove unbound antibody from the membrane, then 10 mL goat anti-rabbit (for polyclonal 1:2000) and anti-mouse (for monoclonal 1:500) IgG peroxidase conjugate (Organdon Teknika, Durham NC 27704) diluted in 3% egg albumin-PBS was added to the membrane and incubated at room temperature for 10 min. Membranes were then washed three times (2 min each) with PBS-Tween to remove excess

peroxidase conjugate. Bound peroxidase was determined using a substrate solution (24 mg 3,3', 5,5'-tetramethylbenzidine and 80 mg of dioctyl sulfosuccinate dissolved in 10 mL ethanol, 30 mL 0.1 M citrate-phosphate buffer, pH 5.0, and 20 μL 30% H_2O_2) at room temperature until optimal color developed. The reaction was stopped by washing with deionized water.

4.3.5 DIFFERENTIAL SCANNING CALORIMETRY

A differential scanning microcalorimeter (DSC) (MC-2, Microcal Inc., Amherst, MA 01002) was used to measure the thermal stability of SM and VI myosins and to determine the effect of pH. The myosin concentration (10 mg/ml) was similar to that used by Wang and Smith (1994b). Myosin and blank solutions (dialysis buffer) were degassed using a vacuum chamber (Nalgene, Fisher Scientific, Pittsburgh, PA 15238) just prior to loading into the DSC. A 2.5 mL Hamilton syringe, with a long needle, was used to fill the lollipop shaped cells (volume 1.24 mL) under low vacuum (ca. 10 cm Hg). The DSC was set at a scan rate of 1°C/min from 25°C to 80°C. Buffer vs. buffer solutions were run before each protein run to obtain a base line for each calculation. VI and SM myosins from 3 different extractions were each tested at pH 5.50 ± 0.10 (SM ultimate pH) and 6.05 ± 0.05 (VI ultimate pH). Cells were cleaned after each run using 5% SDS, 0.1 M EDTA and 3% dithiothreitol in 0.02 M Tris buffer, pH 8.5 by heating at 95°C for 1 hr.

The following parameters were obtained from the heat capacity profile (Cp vs. temperature): calorimetric enthalpy (ΔH_{cal}), van't Hoff enthalpy (ΔH_{vH}), a melting temperature (Tm) at which proteins are 50% denatured, and the cooperative ratio (CR) which was defined as $\Delta H_{vH}/\Delta H_{cal}$

(Privalov and Potekhin, 1986; Tsong et al., 1970). When denaturation is a simple two-state transition, the cooperative ratio is equal to 1 given that ΔH_{vH} is the same as ΔH_{cal}. If the value of CR is greater than 1, there is intermolecular interaction. A CR value below 1 indicates that there is at least one significant intermediate state (Chowdhry and Cole, 1989; Donovan, 1984; Privalov and Potekhin, 1986; Tsong et al., 1970). Myosin molecular mass used in the data analyses was 5.21×10^5 (Yates and Greaser, 1983). The following equation (provided by Microcal) was used to convert the data from calories/degree to calories/degree/mole:

$$N = \frac{\text{Protein concentration (g/L)} \times 1.24 \times 10^{-3}}{\text{Molecular mass (g/mole)}} \quad (L)$$

Each heat capacity-temperature curve was deconvoluted to estimate the number of transition states during myosin unfolding using the procedure described by Freire and Biltonen (1978a,b). These transitions were assumed to be independent two-state type with $\Delta H_{cal} = \Delta H_{vH}$ and the non-linear least squares minimization method was used iteratively until the square sum of residual was lower than 10^{-10}.

4.3.6 TURBIDITY

Protein aggregation was followed by measuring the increase in absorbance at 340 nm in a Cary 3E UV/VIS spectrophotometer (Varian Analytical Instruments, Sunnyvale, CA 94034) equipped with a temperature controller and multicell block at a protein concentration of 5 mg/mL. Preliminary experiments with 2.5, 5 and 10 mg/mL myosin solutions showed that 5 mg/mL gave the best absorbance range during the heating period. Turbidity of VI and SM myosin solutions at pHs of 5.50 ± 0.10 (SM ultimate pH) and 6.05 ± 0.05 (VI ultimate pH) were measured while

heating from 25°C to 80°C at a rate of 1°C/min. The rate of aggregation was calculated as the slope where optical density showed a rapid increase during heating.

4.3.7 DYNAMIC RHEOLOGICAL MEASUREMENTS

A Rheometrics Fluid Spectrometer (RFS-8400, Rheometrics, Inc., Piscataway, NJ 08854) equipped with a 50 mm diameter parallel plate device was used to perform oscillatory dynamic testing of 1% myosin solutions while heating from 25°C to 85°C at 1°C/min rate. Both VI and SM myosin at two different pH's: 5.50 ± 0.10 (SM ultimate pH) and 6.05 ± 0.05 (VI ultimate pH) were studied. The sample cup was heated with a silicone oil circulating system and temperature was controlled by a temperature programmer (MTP-6 Micro-processor, Neslab Instruments, Inc., Newington, NH 03801). Three milliliters of myosin solution were loaded in the sample cup and a gap of 1 - 1.5 mm was set between the upper and lower plates. This volume of myosin covered the upper plate surface. A few drops of corn oil were loaded in the gap between the upper plate and the sample cup to avoid sample dehydration. The instrumental parameters were set to 1% stain and frequency of 1 rad/sec based on preliminary studies.

Storage modulus (G') and loss modulus (G") were recorded every 0.5 min during the controlled heating process. The heating program was as follows: hold for 2 min at 25°C, heat at 1°C/min to 85°C and final hold at 85°C for 2 min. Transition temperatures were defined by the interaction of two regression lines determined from the slope of each rheological transition curve.

4.3.8 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A completely randomized design with three replications was used to compare thermal stability by differential scanning calorimetry, turbidity and dynamic rheological properties of myosin preparations. A two way analysis of variance (ANOVA) was performed to test the significance between myosin isoforms and pH (MSTAT, 1993).

4.4 RESULTS AND DISCUSSION

4.4.1 Myosin extraction and purity

VI and **SM** myosin were extracted three different times. The first time, muscles were ground and mixed in a Waring blendor for two periods of 45 sec each before mixing for 30 min. **VI** myosin precipitated at 41% $(NH_4)_2SO_4$ and **SM** myosin at 35% $(NH_4)_2SO_4$. However, when the blender step was omitted both myosins were precipitated in 41% $(NH_4)_2SO_4$. Thus, the Waring blendor step was omitted in all subsequent extractions.

The precipitate of each muscle formed during the EDTA lowering ionic strength step were different. The **VI** myosin precipitate was compact and fibrous when compared with **SM** precipitate which was finer and looked like swollen gelatin. The yield of myosin as $(NH_4)_2SO_4$ salt precipitated was higher for **SM** (10 -15% from muscle weight) compared with **VI** (6-8% from muscle weight).

In all three extractions, myosin was separated by SDS-PAGE after the $(NH_4)_2SO_4$ precipitation step to evaluate purity (Figure 4.1). One major band corresponding to myosin heavy chain and two other bands below 30 kDa, corresponding to myosin light chains, were observed. Minor contaminating bands at 150 kDa were probably C-protein. Starr and Offer (1971) showed that C-protein was present in a rabbit myosin preparation.

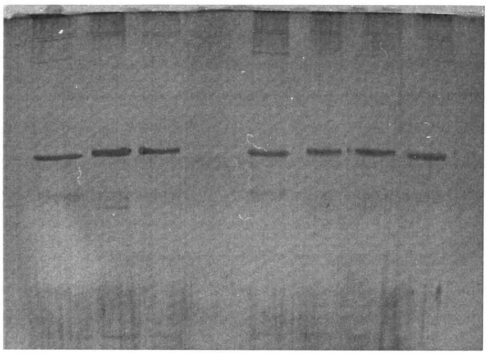


Figure 4.1. Bovine vastus intermedius (**SM**) and vastus intermedius (**VI**) myosin on a sodium dodecyl sulfate-polyacrylamide electrophoretic gel (10%). Lanes: 1, Sigma bovine myosin standard; 2, **VI**; and 3, **SM**.

Callaway and Bechtel (1981) found there were two different C-proteins in bovine skeletal muscle with different electrophoretic mobilities related to muscle fiber type. The red C-protein had higher molecular weight than white C-protein.

4.4.2 Myosin isoform identification

The different myosin heavy chain isoforms in VI and SM myosin preparations were separated using a polyacrylamide and glycerol gradient gel (Sugiura and Murakami, 1990). In the electrophoresis profile (Figure 4.2), the bovine skeletal myosin standard (Sigma) contained three bands corresponding to three myosin heavy chain isoforms related to muscle fiber types I, IIa, and IIb (Billeter et al., 1981). The fastest of the three bands was identified as myosin from type I or slow myosin and the other two bands as fast myosin or type II (Betto et al., 1986; Carraro and Catani, 1983; Sugiura and Murakami, 1990). Bär and Pette (1988) ranked the electrophoretic mobilities of myosin heavy chains (MHC) from slowest to fastest: MHCIIa, MHCIIb and MHCI.

In our myosin preparation, VI myosin contained only one band with the same mobility as the faster band of the myosin standard. However, SM myosin contained three bands. The fastest band was present in SM myosin in very low concentrations as compared with the other two bands. From these results, we suspect that VI myosin was comprised of myosin type I and SM myosin contained type IIa and IIb with a small amount of type I.

To confirm these results, three different Western blots were performed on three identical gradient electrophoresis gels. The proteins were transferred to nitrocellulose membranes and incubated with

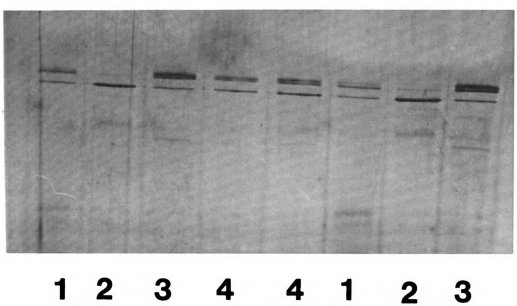


Figure 4.2. Bovine semimembranosus (**SM**) and vastus intermedius (**VI**) myosin run in a gradient sodium dodecyl sulfate-polyacrylamide (5-8%)/glycerol (30-40%) electrophoretic gel. Lanes: 1, Sigma bovine myosin standard; 2, **VI**; 3, **SM**; and 4, equal amounts of **VI** and **SM**.

three different skeletal myosin antibodies: anti-myosin, anti-fast myosin and anti-slow myosin.

The polyclonal anti-myosin reacted with all bands present on the myosin standard and **VI**, **SM**, and **VI** plus **SM** myosin preparations (Figure 4.3). These results confirmed the presence of myosin in our preparations. The anti-fast myosin (Figure 4.4) reacted with the **SM** myosin, but not the **VI** myosin, confirming that **VI** myosin did not contain the fast isoform as suggested from the electrophoresis profile. The anti-slow myosin antibody was from rat and had very poor affinity for bovine muscle. Even so, a weak reaction with the **VI** myosin was noted. No reaction occurred with the other preparations. These results indicated that **VI** myosin contained the slow myosin isoform.

4.4.3 Thermal stability of myosin

Protein unfolding is the first step in the heat-induced gelation of protein. The influence of temperature and pH on the thermal stability of myosin from **VI** (red muscle) and **SM** (white muscle) were followed using a differential scanning calorimeter (Figure 4.5). In general, the heat capacity curves of the four treatments contained two major peaks, one before and one after 55°C.

VI myosin at pH 6.05 (**VI** ultimate pH) began to unfold at 32°C. When heated from 25°C to 80°C, three peaks were observed in the heat capacity curve at 53°, 57°, and 65°C. The calorimetric enthalpy (ΔH_{cal}) was 1496 kcal/mol and van't Hoff enthalpy (ΔH_{vH}) was 44 kcal\mol with a cooperative ratio (CR = ΔH_{vH}/ ΔH_{cal}) of 0.03 (Table 4.1). A CR below 1 implied that there was more than one domain in **VI** myosin at pH 6.05.

When the pH of **VI** myosin was adjusted to 5.50 (**SM** ultimate pH),

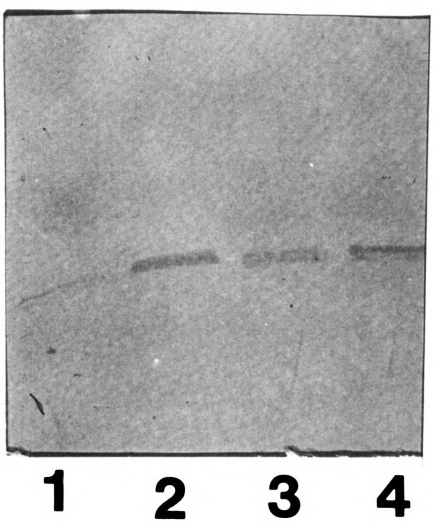


Figure 4.3. Western blot of myosin visualized using anti-myosin polyclonal antibodies. Lanes: 1, Sigma bovine myosin standard; 2, vastus intermedius- **VI**; 3, semimembranosus- **SM**; and 4, equal amounts of **VI** and **SM**.

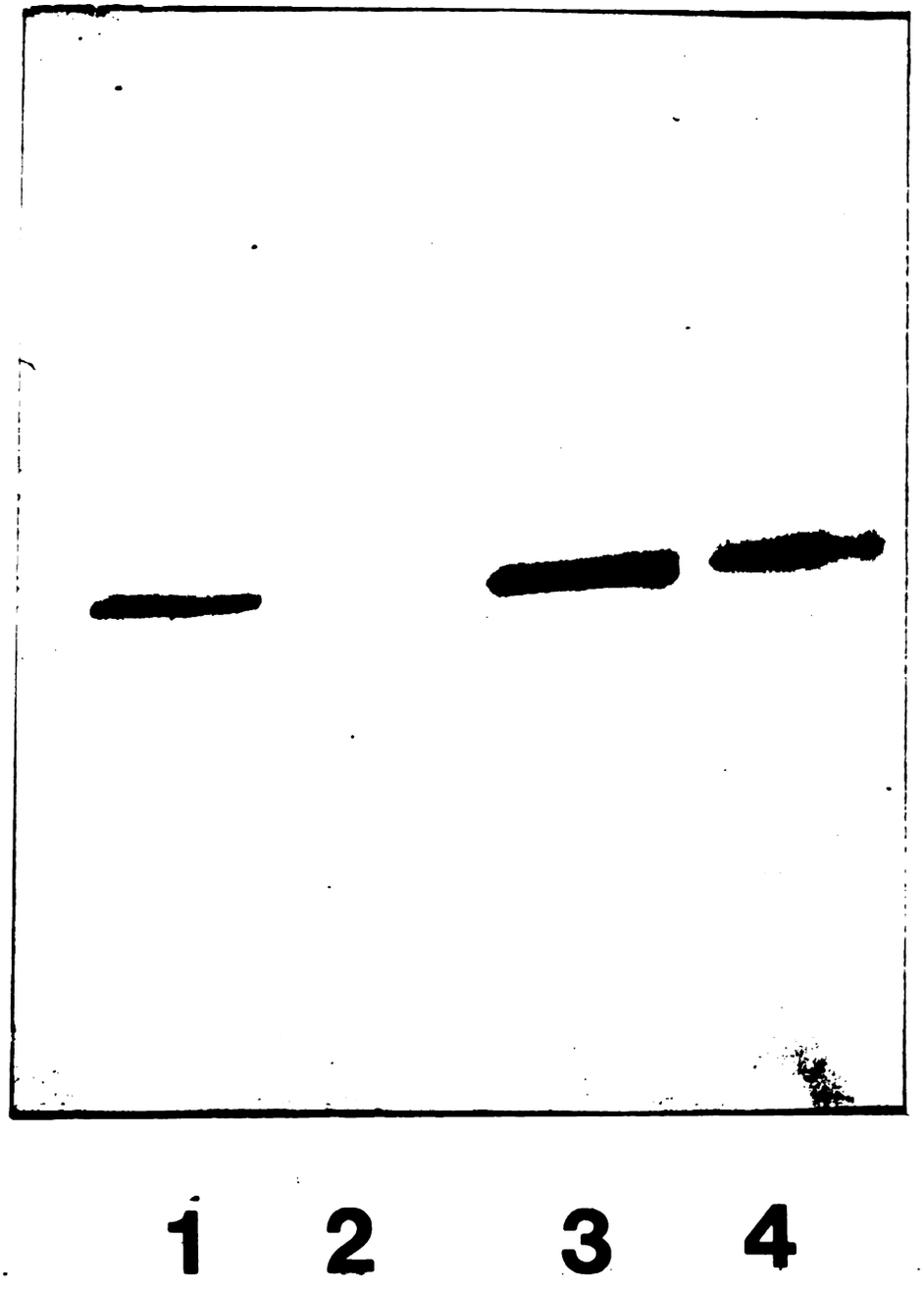


Figure 4.4. Western blot of myosin visualized using anti-fast myosin monoclonal antibody. Lanes: 1, Sigma bovine myosin standard; 2, vastus intermedius- VI; 3, semimembranosus-SM; and 4, equal amounts of VI and SM.

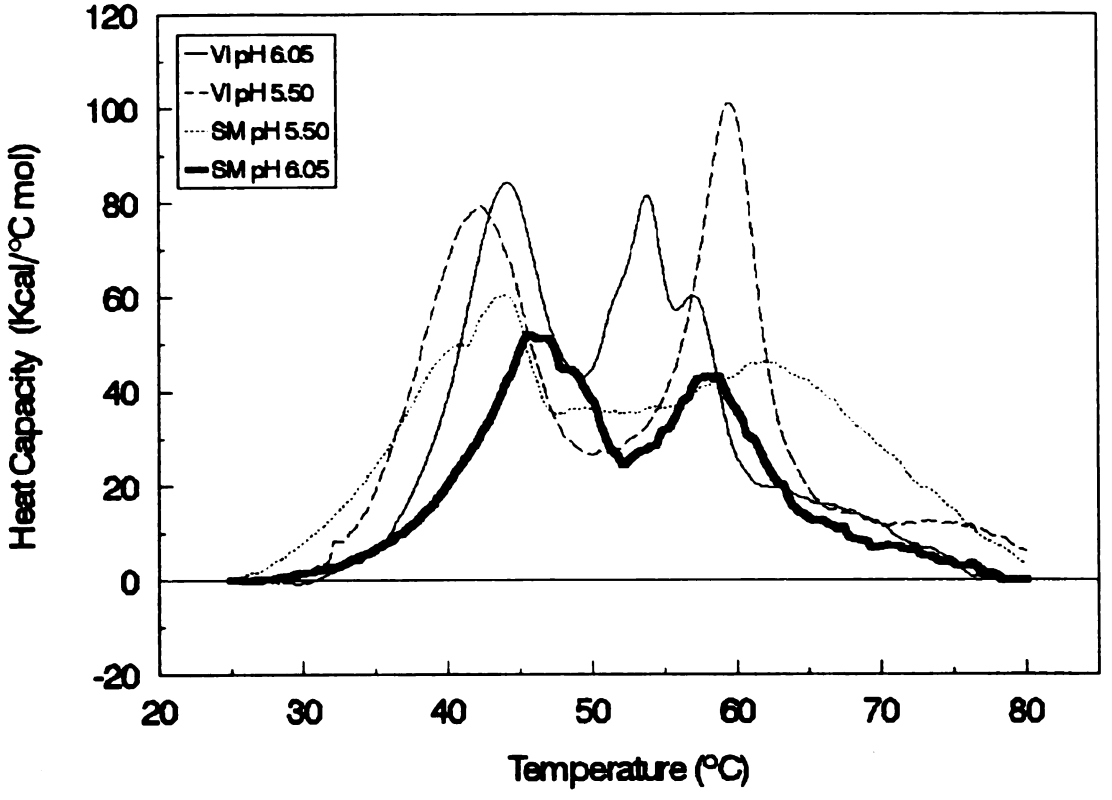


Figure 4.5. Differential scanning calorimetry endotherms of bovine semimembranosus (SM) and vastus intermedius (VI) myosin solutions. One percent (10 mg/ml) of myosin in 0.6 M NaCl and 50 mM sodium phosphate buffer at pH 5.50 and 6.05. Scan rate is 1°C/min.

Table 4.1 Enthalpies and transition temperature of bovine vastus intermedius (SM) and vastus intermedius (VI) myosin endotherm in 0.6 M NaCl, 0.05 M sodium phosphate buffer

Muscle	pH	ΔH_{cal}[ab] (kcal/mol)	Transition Temperature (°C) To[c]	T1[d]	T2[d]	T3[d]	Tm[e]
SM	5.50	1972[f] ±321	25 43 ±1.7	50 ±0.6	62 ±0.1	43 ±0.1	±0.7
SM	6.05	1416[g] ±503	31 ±0	46 ±0.2	58 ±0		50 ±7.2
VI	5.50	1744[f] ±82	30 ±1.3	42 ±0.4	58 ±0.9		54 ±9.9
VI	6.05	1496[g] ±57	32 ±2.0	53 ±0.1	57 ±0.5	65 ±0.1	51 ±5.7

[ab] ΔH_{cal} is calorimetric enthalpy mean of three replicates ± standard deviation.
[c] To is the temperature where myosin started to unfold.
[d] Tn is the temperature where n transition or peak occurred.
[e] Tm is the melting temperature or where half the molecule was unfolded.
[f,g] Figures with different superscripts are significant different at $p>0.05$.

myosin began to unfold at 30°C. VI myosin at pH 5.5 began to unfold 2°C before the same protein at 6.05. The heat capacity profile presented two major peaks at 42 and 58°C. The ΔH_{cal} of VI myosin was 250 kcal/mol greater at pH 5.5 than at pH 6.05 (Table 4.1).

SM myosin at pH 5.50 (SM ultimate pH) began to unfold at 25°C which was the lowest temperature of the four treatments. The SM myosin heat capacity profile showed three peaks at 43.3, 50, and at 62°C with two shoulders at 41 and 58°C. The ΔH_{cal} was the highest among all the treatments at 1972 kcal/mol. The ΔH_{vH} was lowest at 30 kcal/mol (Table 4.1).

When the pH was adjusted to 6.05 (VI ultimate pH), SM myosin did not start to unfold until about 31°C. The endothermic curve showed two peaks at 46 and 58°C with two shoulders at 44 and 50°C. The ΔH_{cal} of SM myosin at pH 6.05 was 556 kcal/mol less than the calorimetric enthalpy at pH 5.50 (Table 4.1).

When VI and SM myosin were compared at the same pH (Figure 4.5), VI and SM myosins started to unfold almost at the same temperature at pH 6.05. At pH 5.50, VI myosin had sharp transition peaks, whereas SM myosin had broad short peaks. The ΔH_{cal} of VI and SM were not significantly different at the same pH, although the transition temperature range of SM myosin was higher at each pH compared to VI myosin.

The heat capacity profile of VI and SM myosins were broader when the pH decreased, lowering the temperature of the first transition and increasing the temperature for the last transition. Myosin from both muscles were less stable at pH 5.50 given that the first transition peak of each muscle occurred at a lower temperature than at pH 6.05. The

second major peak occurred at a higher temperature at pH 5.50. Egelandsdal et al. (1994) found the same behavior for bovine masseter at pH 5.0, 6.0, and 7.0. Xiong and Brekke (1990a) found transition temperatures (Tm) of SSP of chicken breast and leg was lower at pH 5.50 than pH 6.0 in both muscle by almost 5°C. Bertazzon and Tsong (1990) found a similar trend with pH on the thermal unfolding of rabbit fast myosin rod.

4.4.3.1 Deconvolution of myosin endotherms

Ten different two-state transitions were obtained when the endotherm curve of **SM** and **VI** myosins were deconvoluted. The same number of domains were obtained for Wang and Smith (1994a) in chicken breast myosin. The transition temperature and enthalpy of each domain are presented in Tables 4.2 - 4.5 for each combination of muscle and pH.

The deconvoluted peaks for **VI** myosin at pH 6.05 and pH 5.50 are shown in Figures 4.6 and 4.7, respectively. **VI** myosin unfolded over a 37°C temperature range at pH 5.50 as compared to a 30°C temperature range at pH 6.05. The temperature for the unfolding of the first domain of **VI** myosin at pH 6.05 was approximately 5°C higher and the last deconvoluted domain was approximately 2°C lower than **VI** myosin at pH 5.50. The enthalpy value of the ten transitions at pH 5.50 were higher than those of **VI** myosin at pH 6.05.

In the case of **SM** myosin at pH 5.50, the transition temperature range of 39°C was the broader range of the four treatments. The heat profile and deconvoluted curve for **SM** myosin at pH 5.50 and 6.05 are shown in Figures 4.8 and 4.9, respectively. When the pH of **SM** myosin was adjusted to pH 6.05, the molecule unfolded over a 28°C temperature

Table 4.2 Temperature and calorimetric enthalpy of bovine vastus intermedius myosin at pH 6.05 from differential scanning calorimetry (DSC) deconvoluted peaks when heated from 25 to 80°C at 1°C/min

Peak	T (°C)[a]	ΔH_{vH}[b] = ΔH_{cal}[c] (kcal/mol)	Turbidity (°C)[d]	Onset gelation[e] temperature
1	40.0 ± 0.8	116.0 ± 3.2		
2	43.2 ± 0.9	154.7 ± 10.1		
3	45.1 ± 0.8	148.4 ± 23.7		
4	46.8 ± 2.0	162.2 ± 7.1		
5	48.4 ± 0.6	127.8 ± 14.5		
6	52.5 ± 1.0	162.1 ± 61.1	53	
7	54.1 ± 0.6	181.3 ± 64.3		
8	57.7 ± 0.2	195.6 ± 6.8	60	57.2
9	64.2 ± 1.1	125.5 ± 7.4		
10	70.7 ± 1.3	110.7 ± 9.7		
10-1[f]	30.7 ± 0.6			

[a] Mean ± standard deviation of unfolded temperature of each domain from three replicates.

[b] ΔH_{vH} van't Hoff enthalpies.

[c] ΔH_{cal} calorimetric enthalpies.

[d] Temperature where the turbidity starts increasing and where occurred higher OD.

[e] The onset gelation temperature where G'=10 Pa by dynamic measurements.

[f] 10-1 is the temperature range for deconvoluted peaks.

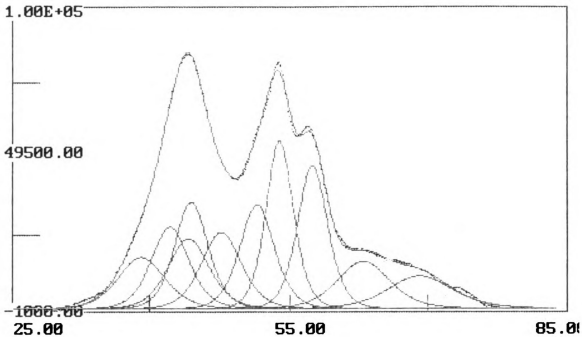


Figure 4.6. Heat capacity profile and deconvoluted peaks of bovine vastus intermedius myosin in 0.6 M NaCl, 50 mM sodium phosphate at pH 6.05. Scan rate is 1°C/min. The theoretical endotherm and deconvoluted peaks are represented by solid line and experimental data by dotted line.

Table 4.3 Temperature and calorimetric enthalpy of bovine vastus intermedius myosin at pH 5.50 from differential scanning calorimetry (DSC) deconvoluted peaks when heated from 25 to 80°C at 1°C/min

Peak	T (°C)[a]	ΔH_{vH}[b] = ΔH_{cal}[c] (kcal/mol)	Turbidity (°C)[d]	Onset gelation[e] temperature
1	35.0 ± 3.0	123.9 ± 13.3	25	
2	39.0 ± 1.5	171.8 ± 10.8		37.5
3	41.9 ± 0.9	193.1 ± 10.9		
4	44.4 ± 0.5	185.6 ± 22.5	45	
5	48.4 ± 0.4	141.6 ± 20.6		
6	53.4 ± 0.4	151.7 ± 23.7		
7	57.1 ± 0.5	196.4 ± 19.2		
8	59.7 ± 0.2	236.7 ± 28.4		
9	63.7 ± 1.6	151.3 ± 14.5		
10	72.5 ± 1.5	120.1 ± 11.9		
10-1[f]	37.5 ± 1.6			

[a] Mean ± standard deviation of unfolded temperature of each domain from three replicates.

[b] ΔH_{vH} van't Hoff enthalpies.

[c] ΔH cal calorimetric enthalpies.

[d] Temperature where the turbidity starts increasing and where occurred higher OD.

[e] The onset gelation temperature where G'=10 Pa by dynamic measurements.

[f] 10-1 is the temperature range for deconvoluted peaks.

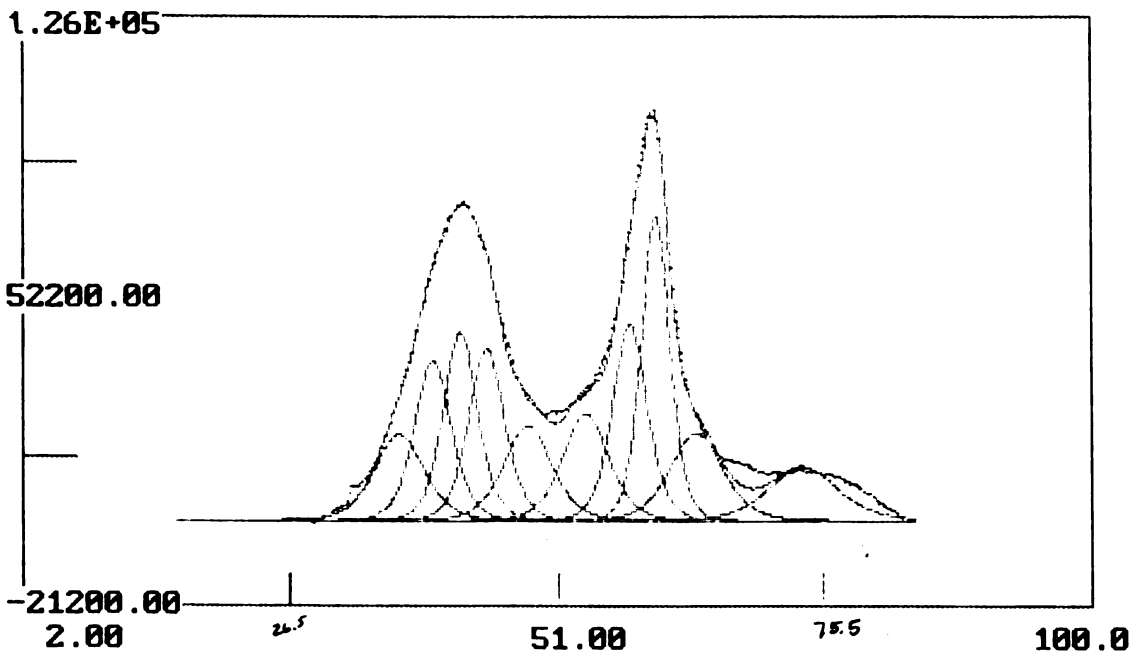


Figure 4.7. Heat capacity profile and deconvoluted peaks of bovine vastus intermedius myosin in 0.6 M NaCl, 50 mM sodium phosphate at pH 5.50. Scan rate is 1°C/min. The theoretical endotherm and deconvoluted peaks are represented by solid line and experimental data by dotted line.

Table 4.4 Temperature and calorimetric enthalpy of bovine semimembranosus myosin at pH 5.50 from differential scanning calorimetry (DSC) deconvoluted peaks when heated from 25 to 80°C at 1°C/min

Peak	T (°C)[a]	ΔH_{vH}[b] = ΔH_{cal}[c] (kcal/mol)	Turbidity (°C)[d]	Onset gelation temperature[e]
1	33.6 ± 3.0	130.3 ± 18.8		
2	38.5 ± 0.4	183.2 ± 23.3	39	
3	42.0 ± 0.1	204.2 ± 30.9		
4	44.8 ± 0.2	203.0 ± 16.1		
5	49.4 ± 0.2	181.3 ± 19.1		
6	53.9 ± 0.3	179.0 ± 19.0		54
7	58.3 ± 0.3	190.5 ± 18.1	60	
8	62.5 ± 0.2	199.1 ± 16.7		
9	66.9 ± 0.2	186.2 ± 18.1		
10	72.3 ± 0.4	152.1 ± 17.0		
10-1[f]	38.7 ± 0.5			

[a] Mean ± standard deviation of unfolded temperature of each domain from three replicates.

[b] ΔH_{vH} van't Hoff enthalpies.

[c] ΔH cal calorimetric enthalpies.

[d] Temperature where the turbidity starts increasing and where occurred higher OD.

[e] The onset gelation temperature where G'=10 Pa by dynamic measurements.

[f] 10-1 is the temperature range for deconvoluted peaks.

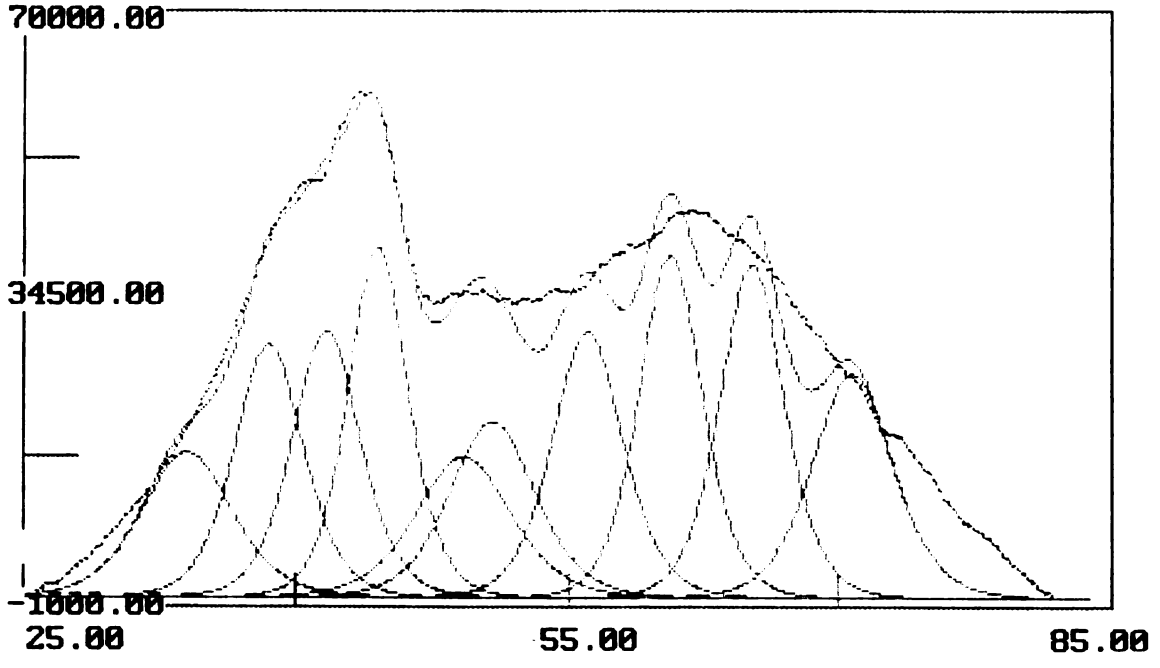


Figure 4.8. Heat capacity profile and deconvoluted peaks of bovine semimembranosus myosin in 0.6 M NaCl, 50 mM sodium phosphate at pH 5.50. Scan rate is 1°C/min. The theoretical endotherm and deconvoluted peaks are represented by solid line and experimental data by dotted line.

Table 4.5 Temperature and calorimetric enthalpy of bovine semimembranosus myosin pH 6.05 from differential scanning calorimetry (DSC) deconvoluted peaks when heated from 25 to 80°C at 1°C/min

Peak	T (°C)[a]	ΔH_{vH}[b] = ΔH_{cal}[c] (kcal/mol)	Turbidity (°C)[d]	Onset gelation[e] temperature
1	40.3 ± 2.2	114.4 ± 49.1		
2	43.3 ± 1.6	134.1 ± 64.8		
3	46.1 ± 0.6	186.4 ± 42.5		
4	49.2 ± 0.9	138.6 ± 61.9	49	
5	50.2 ± 1.6	107.2 ± 82.4		50
6	51.8 ± 2.6	133.9 ± 54.0		
7	56.2 ± 0.7	166.9 ± 43.6		
8	59.1 ± 0.8	188.0 ± 48.7	60	
9	62.7 ± 1.7	135.6 ± 39.4		
10	68.5 ± 2.8	101.5 ± 25.2		
10-1[f]	28.2 ± 3.4			

[a] Mean ± standard deviation of unfolded temperature of each domain from three replicates.

[b] ΔH_{vH} van't Hoff enthalpies.

[c] ΔH cal calorimetric enthalpies.

[d] Temperature where the turbidity starts increasing and where occurred higher OD.

[e] The onset gelation temperature where G'=10 Pa by dynamic measurements.

[f] 10-1 is the temperature range for deconvoluted peaks.

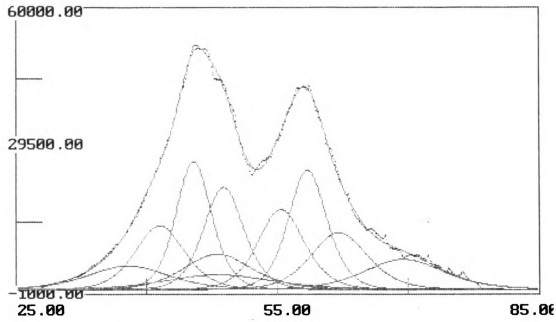


Figure 4.9. Heat capacity profile and deconvoluted peaks of bovine semimembranosus myosin in 0.6 M NaCl, 50 mM sodium phosphate at pH 6.05. Scan rate is 1°C/min. The theoretical endotherm and deconvoluted peaks are represented by solid line and experimental data by dotted line.

range, the lowest value of the four treatments. Similar to **VI** myosin, the first domain of **SM** myosin unfolded at a lower temperature at pH 5.50 (ca. 6.6°C) and the last domain unfolded at a higher temperature (ca. 4°C) than those of **SM** myosin at pH 6.05. **SM** myosin domain enthalpies at pH 5.5 were higher than those of **SM** myosin at pH 6.05.

The first three deconvoluted domains of **VI** and **SM** myosin at pH 6.05 were similar. However, the last unfolded domain occurred at a higher temperature (ca. 2°C) for **VI** than **SM**. At pH 5.50, **VI** and **SM** myosins had different behavior. Early domains of **VI** myosin appeared more stable as indicated by a higher temperature. However, the latter domains in **SM** were the more stable.

4.4.4 Turbidity

Protein aggregation was followed by measuring the increase in optical density (OD, Figure 4.10). The rate of aggregation has been considered to influence the degree of organization in the gel structure (Ferry, 1948; Ziegler and Foegeding, 1990). The bovine myosin molecule aggregates on heating to form two types of gels (fine-stranded or globular aggregates), depending on the solution ionic strength (Hermansson et al., 1986).

VI myosin at pH 6.05 (red muscle ultimate pH) showed a constant OD until 53°C. After this temperature, **VI** myosin OD increased very rapidly between 54-57°C at a rate of 0.3 OD/°C. This aggregation rate was the highest among all treatments. The highest OD was at 60°C followed by second small transition at 65°C. The OD of **VI** myosin decreased during the rest of the heating period.

When the pH of **VI** myosin was adjusted to 5.50 (white ultimate pH),

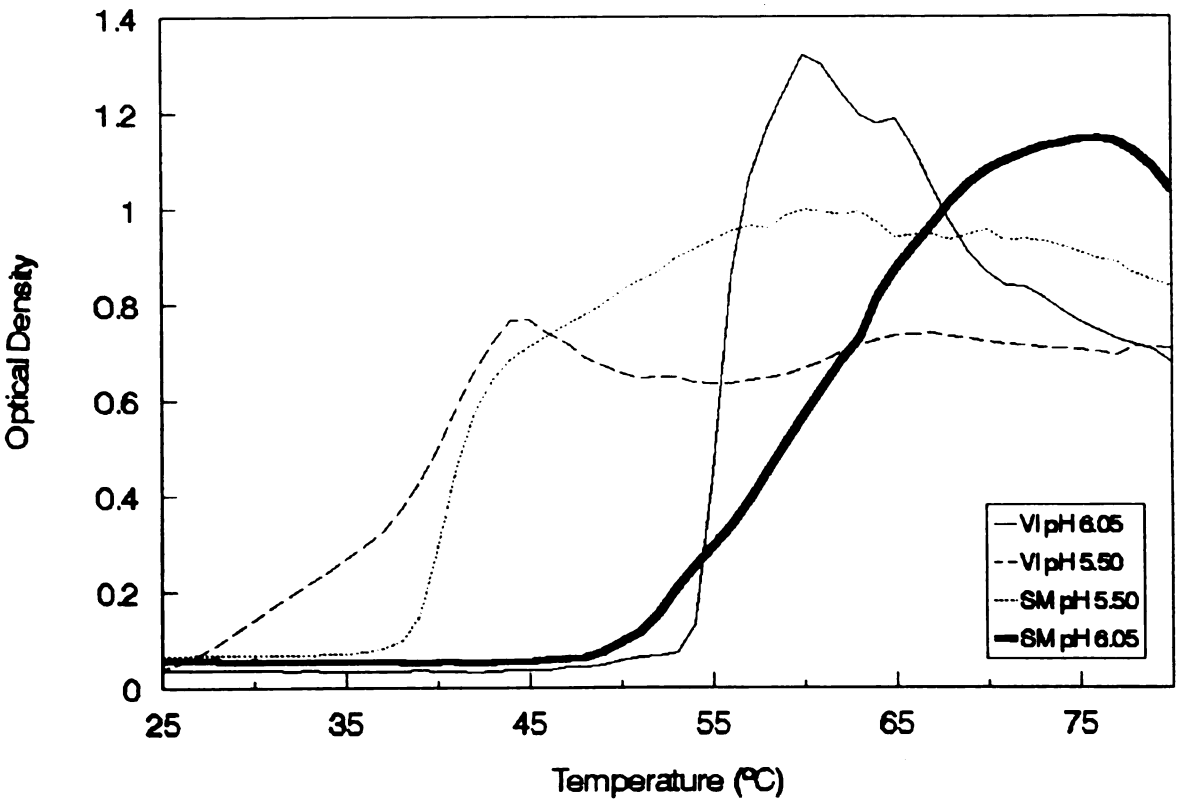


Figure 4.10. Turbidity of bovine semimembranosus (SM) and vastus intermedius (VI) myosin solution (5 mg/mL) in 0.6 M NaCl, 50 mM sodium phosphate at pH 5.50 and 6.05. Absorbance measured at 340 nm with a scan rate 1°C/min.

OD started to increase at 25°C. Turbidity increased slowly from 25-37°C (0.02 OD/°C) and more rapidly from 37-45°C (0.06 OD/°C). The optical density for **VI** at pH 5.50 showed two peaks at 45°C and 67°C. For **VI** at pH 5.50, the highest turbidity was at a lower temperature (45°C) and the magnitude was lower than **VI** myosin at pH 6.05. The first transition was at a lower temperature and the second at a higher temperature when pH was decreased.

Optical density for **SM** myosin at pH 5.50 was constant up to 39°C, followed by a rapid increase in turbidity up to 43°C (0.12 OD/°C). The rate of aggregation was lower between 43 - 60°C (0.02 OD/°C), where the first transition and highest OD were observed.

When **SM** myosin pH was adjusted to 6.05 (ultimate pH of **VI**), OD began to increase at 49°C and followed a sigmoidal type of curve to 76°C. The fastest rate of aggregation was observed between 49 - 64°C (0.05 OD/°C) followed by a slower rate of aggregation until a peak at 76°C. The OD decreased slowly during the rest of heating period. The OD profile for **SM** myosin showed one transition. The transitions for **SM** at pH 6.05 occurred at a higher temperature and the OD was higher than **SM** at pH 5.50.

The initial indication of aggregation (change OD > 0.01) of **VI** and **SM** myosin occurred at a higher temperature at pH 6.05 than at pH 5.50. **VI** myosin began to aggregate 4°C higher than **SM** myosin at pH 6.05. The range of temperature where aggregation occurred was broader for **SM** myosin (by 20°C) than for **VI** myosin at pH 6.05. **VI** myosin underwent two transitions at lower temperatures (60°C and 65°C) than **SM** myosin which only presented one transition at 76°C. Similar to the results in bovine semimembranosus myosin of Hermansson et al. (1986) two types of gels

were observed at the two pHs (5.50 and 6.05). Moreover, the initial solution of myosin at pH 5.50 was more turbid than the solution at pH 6.05. Egelandsdal et al. (1994) found the optical density of bovine cutaneus trunci (fast) and masseter (slow) solution at pH 5.50 were higher than pH 6.05.

However, at pH 5.50 the OD of **VI** myosin started to increase 14°C lower than **SM** myosin. **VI** and **SM** myosin presented two transition peaks, but **VI** myosin transitions (45°C and 67°C) were again at lower temperatures than for **SM** myosin (60 and 70°C). The aggregation rate was higher for **SM** than **VI** myosin. The rates of maximum aggregation were different for the four treatments. The aggregation rate of **VI** (pH 6.05) and **SM** (pH 5.50) myosin at their ultimate pH were similar and higher than when each muscle was adjusted to the pH of the other muscle. The temperature range where OD kept increasing were broader when pH was adjusted to the ultimate pH of the other muscle. The OD peak was higher for **VI** than **SM** myosin at pH 6.05, but this trend reversed when compared at pH 5.50. These results were also reported by Morita et al. (1987) for chicken breast and leg myosin.

4.4.5 Viscoelastic properties

Storage modulus (G', Figure 4.11), loss moduli (G", Figure 4.12), and tangent delta (tan δ, Figure 4.13) were measured during heating to follow the gelling process of **VI** and **SM** myosin. An increase of G' (storage modulus) and a decrease of tan δ indicated the formation of a solid type material and suggested a change from a protein solution to gel (Hamann, 1991). Gelation onset was defined when G' was equal to 10 Pa.

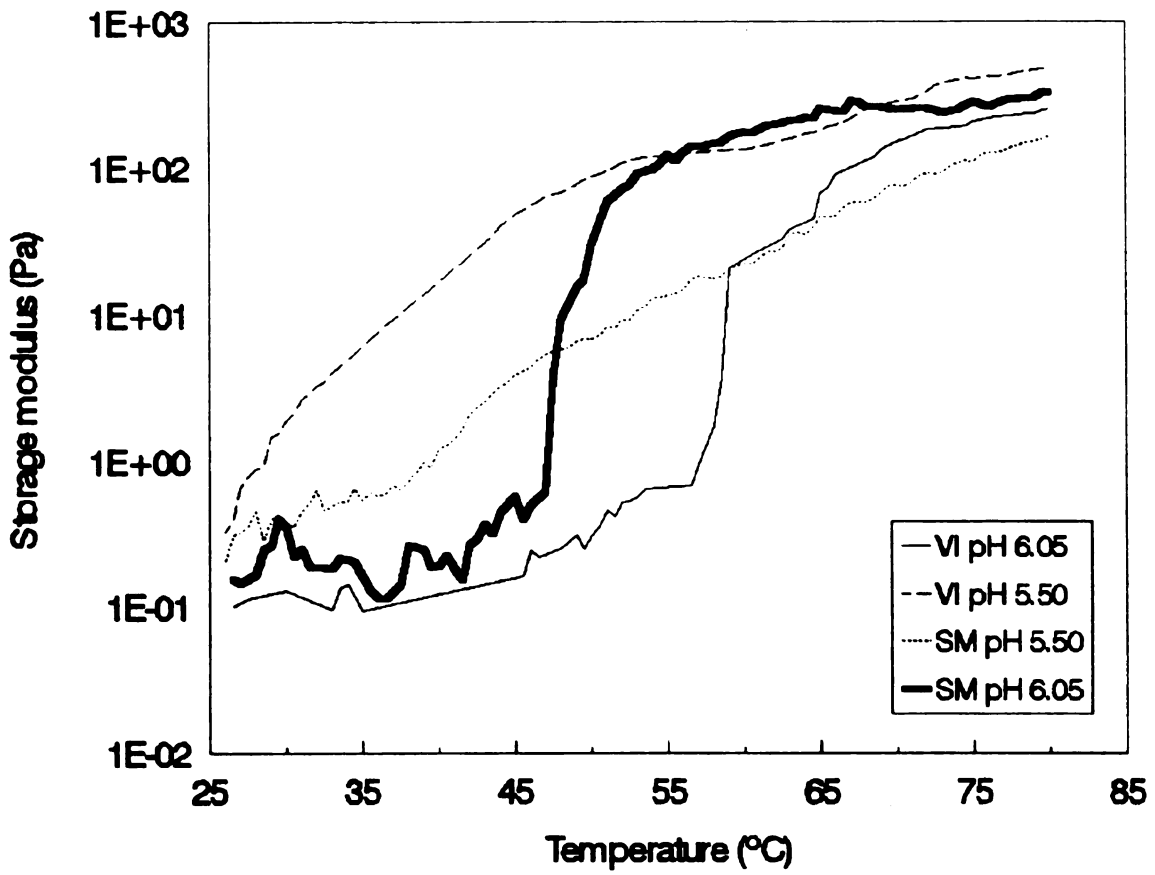


Figure 4.11. Storage modulus (G') of 10 mg/ml myosin from bovine semimembranosus (SM) and vastus intermedius (VI) heated 1°C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer at pH 5.50 and 6.05.

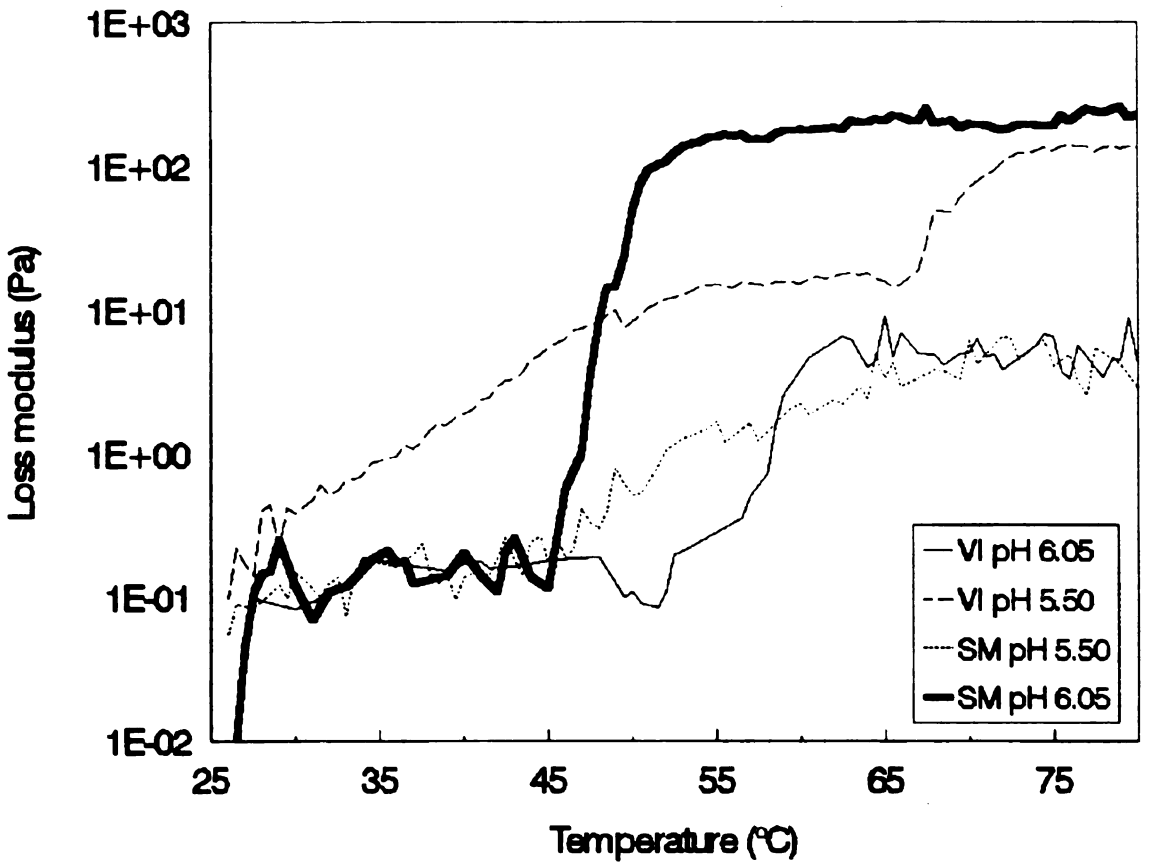


Figure 4.12. Loss modulus (G") of 10 mg/ml myosin from bovine semimembranosus (SM) and vastus intermedius (VI) heated 1°C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer at pH 5.50 and 6.05.

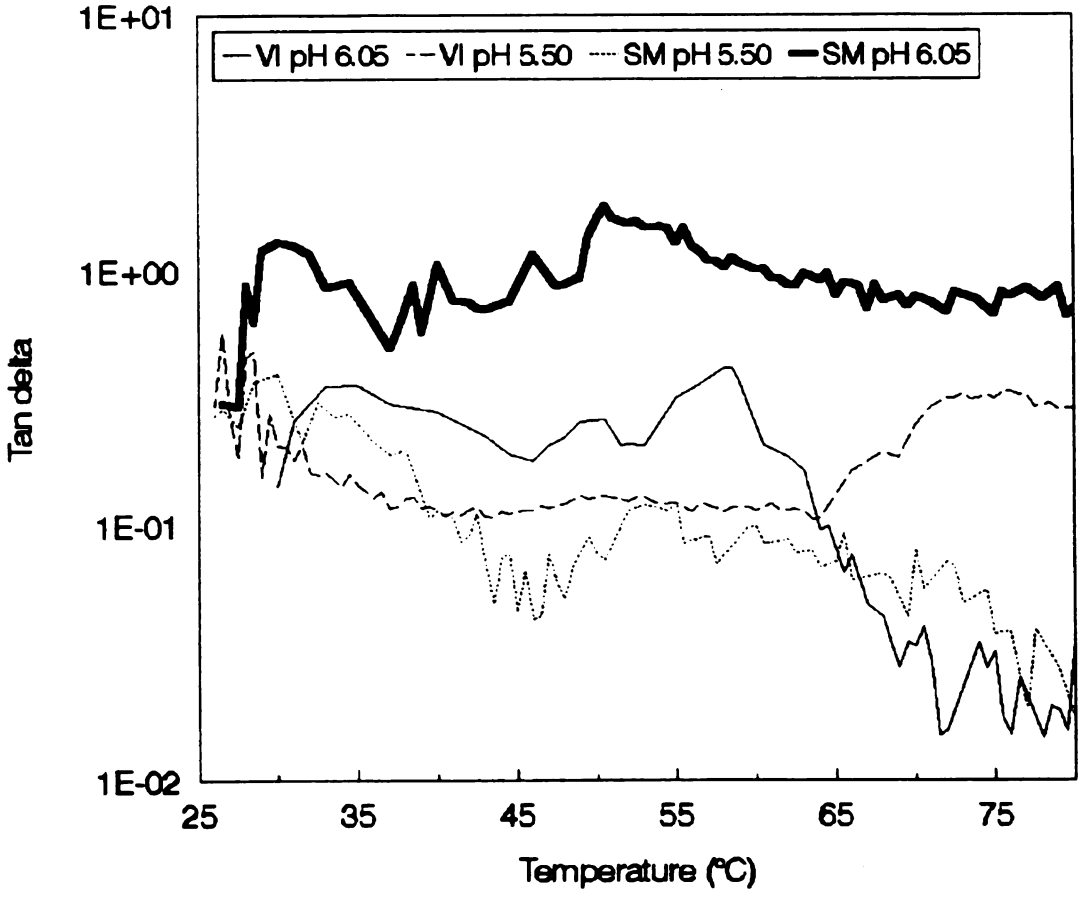


Figure 4.13. Tangent delta (tan δ) of 10 mg/ml myosin from bovine semimembranosus (SM) and vastus intermedius (VI) heated 1°C/min in 0.6 M NaCl, 50 mM sodium phosphate buffer at pH 5.50 and 6.05.

Storage modulus for VI myosin at pH 6.05 was inconsistent until about 57°C when G' increased rapidly to 58°C followed by a slower increase during the rest of the heating period. Initial G' was about 0.1 Pa and the final G' at 80°C was 300 ± 55 Pa. The onset of gelation (G' =10 Pa) occurred at 57.2°C. Loss modulus and G' followed a similar profile during heating. However, the final G" was only 3.2 Pa. Tan δ for this solution decreased after 55-58°C. This decrease in tan δ indicated the development of solid-like behavior suggesting gel matrix formation.

When the pH of VI myosin was adjusted to 5.50 (SM ultimate pH), the profile of G' changed. There was a steady increase in G' during the entire heating period with an small change in slope at 50°C. The onset of gelation occurred at a lower temperature of 34.5°C. G' and G" at 80°C were higher than VI myosin at pH 6.05. Tan δ of VI myosin decreased on heating above 50°C.

The G' of SM myosin at pH 5.50 increased steadily during the heating period. Initial G' was about 0.1 Pa. The onset of gelation occurred at 54°C and the G' at 80°C was 149 Pa. The initial G" value was similar to G', but was lower than G' during the heating period. The G" at 80°C was 6 Pa. The initial value of tan δ was very low but decreased at temperatures above 55°C.

At pH 6.05 (VI ultimate pH), SM myosin G' was irregular until 46°C, where a sharp increase of G' for 8°C followed by an small increase during the rest of heating period. The initial G" was about 0.1 Pa and the G' at 80°C was 225 Pa. The onset of gelation occurred when SM myosin reached 50°C. The G" rheogram was similar to that of G'. The final G" at 80°C was about 100 Pa and lower than G'.

Gel matrix formation of both muscles occurred at a lower temperature at pH 5.50, which agreed with aggregation. The rheogram of storage moduli was similar for both muscles at the same pH. The onset gelation temperature and final storage moduli differed with pH. **VI** myosin formed a gel at a lower temperature than **SM** at pH 5.50, which agreed with the lower thermal stability and faster aggregation observed at this pH. However, this trend was reversed at pH 6.05. **SM** myosin at pH 6.05 presented a lower temperature for the onset of gelation and the final G' at 80°C was higher than **VI** myosin at pH 6.05.

4.5 Conclusions

The differences in heat-induced gelation properties of **VI** and **SM** myosin were influenced by protein isoform and ultimate pH in a synergistic manner. However, pH had greater influence on the three steps of gelation: unfolding, aggregation, and gel matrix formation. In conclusions of this study try to explain the myosin gelation throughout each step of the heat-induced followed by DSC, turbidity and dynamic testing at each pH.

At pH 5.50 the heat stability of **VI** myosin was lower than **SM** myosin and the formation of filaments was clear by the turbidity of the solution and the lower temperature where optical density started to increase. The effect of this low pH was greater in **VI** myosin than **SM** myosin as indicated by the lower initial temperature of increasing turbidity and the onset gelation temperature compared with those for **SM**.

When **SM** and **VI** myosins are compared at pH 6.05, some of the myosin domains need to unfold before any aggregation happened from the turbidity results. Even though both myosins started to unfold at the

same temperature, the aggregation step requires unfolding of six domains for **VI** myosin, while only four of them were needed for **SM** myosin. The onset gelation temperature in both cases occurred after temperature where aggregation was initiated.

In agreement with literature data, **SM** myosin appeared to be less heat stable than **VI** myosin. However, temperature range from the initial sign of aggregation to the temperature of maximum OD was bigger for **SM** myosin than **VI** myosin. The information can be used in the meat industry to manipulate pH and temperature and time of processing to obtain similar gelation with both muscle types.

CONCLUSIONS

The most important conclusion gathered from this research on salt soluble proteins and myosin was that both fiber type and ultimate pH influenced the gelation properties of myosin. The interrelationship of both of these factors and their influence on gelation were more visible in the myosin system than in the SSP system, that contained many proteins.

In the first study, VI and SM muscle presented different compositions; and pH influence were different in both muscles. The difference in pH range where the solubility of SSP decreased could explain the different type of gel formed at pH 5.50 in both muscles. By adjusting the pH of SM (white) to 6.05 (VI-red ultimate pH), SSP gels were shown to have better water holding capacity then SM gels at their ultimate pH (5.50). However, when VI SSP was adjusted to pH 5.50, there was a decrease in gel water holding capacity. Similar G' profiles were observed at pH 6.05 for both VI and SM during heating. However, SM SSP was less heat stable than VI SSP, as indicated by an earlier increase in G' at both pH. In general, SSP from SM (white) muscle formed stronger gels than those from VI at pH 5.50 and 6.05. Gel properties of SM were not significantly different at either pHs. In the case of VI SSP, gel properties such as gel strength and water holding properties, appeared to increase when the pH was adjusted to the ultimate pH of SM, but this could be an artifact from the higher final protein concentration of

these gels due to their decreased water holding capacity.

In the myosin study, SM myosin was shown to be primarily of the type II isoform, since it contained the slower of the two bands in the gradient SDS-PAGE gel and was recognized in a Western blot by antibodies against fast bovine skeletal myosin. The VI myosin was identified as type I (red myosin isoform) due to its faster migration in the same SDS-PAGE gel as compared to the type II isoform and its non-reactivity with anti-fast myosin antibody in the Western blot. A difference was seen in the thermal stability of different myosin isoforms which could not be compensated for by adjusting the pH of the myosin solutions. SM myosin was less heat stable than VI, as indicated by the lower temperature at which the endotherm rose from the baseline. The calorimetric enthalpies were higher at pH 5.50 than pH 6.05. The cooperativity ratio was below 1 at both pHs. In thermal scanning experiments, VI and SM myosin presented three transitions at pH 6.05 and only two at pH 5.50. The last transition, related to the unfolding of LMM, was more stable in SM than VI myosin. Ten domains were obtained when endotherms were deconvoluted. The first three domains occurred at lower temperatures for SM than VI myosin. The aggregation of both myosins started (OD>0.02 from initial OD) at lower temperatures at pH 5.50 than 6.05, and VI myosin was more sensitive to pH than SM. Aggregation appeared to follow different mechanisms for the two fiber types and pHs, given the different shapes of turbidity curves. The aggregation rates were different for VI and SM at pH 5.50 and 6.05 and two different types of gels were formed, depending on pH. In the aggregation step, there were more similarities in the turbidity profile between myosin isoforms at their own ultimate pH then when isoforms were compared at the same pH.

Even though there were temperature differences at which the gel network was formed, isoforms at the same pH presented similar storage modulus rheograms. The gelation onset occurred at a lower temperature for myosins at pH 5.50 than 6.05, which was in agreement with the aggregation results and thermal stability of **VI** and **SM** myosin. The G' at 80°C for **VI** was higher than **SM** at pH 5.50, but the reverse was seen at pH 6.05.

Given that muscle fiber type was responsible for differences in the thermally induced gelation properties of SSP and myosin, the current standards of protein content formulation used by processors could result in undesirable properties when muscles containing different fiber types are used. Using this information, meat processors should select cuts containing specific fiber types for use in individual products and adjust pH and thermal history to better maximize yield and product quality.

FUTURE RESEARCH

The heat induced gelation of salt soluble proteins, mainly myosin, has been shown to be very important in the yield and texture of processed meat products. Given the decline in the consumption of fresh beef, development of new processed meat products using beef would be highly recommended. Most bovine muscles are a combination of white and red fibers, an important factor that needs to be considered during the development of new products. This study has demonstrated the influence of fiber type on thermally induced gelation of SSP and myosin. It would be beneficial to examine the effect of differing amounts and origin of fiber types, as well as pH, on product quality of processed meat products.

If the final product quality appears to be affected by fiber type, additional research into fiber type content of muscles would be needed. In addition, the content of fiber types in different muscles has been shown to vary with age, nutritional status, gender and physical activities. Therefore the development of a quick and simple assay, such as an immunoassay, capable of quantifying specific fiber types would be useful.

The thermal unfolding of different myosin isoforms were different, but the deconvoluted endotherms revealed the same ten two-state transitions for both fiber types, but at slightly different temperatures. Each domain corresponds to a portion of the myosin

molecule. Identifying specific differences among myosin isoforms would aid in understanding the differences in gelation. Therefore, thermal scanning and turbidity testing of myosin subfragments would be helpful in understanding the unfolding and aggregation of myosin.

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