

## FUNCTIONALITY OF MYOFIBRILLAR PROTEINS FROM CATTLE AT DIFFERENT MATURITIES

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**KEY WORDS:** Beef, semimembranosus muscle, fiber type, myofibrillar protein, gelation

## ABSTRACT

Myofibrillar proteins isolated from semimembranosus muscle of 10 (I)-, 16 (II)- and 22 (III)-mon old cattle were evaluated for solubility and gelation properties in 0.6 M NaCl solution. Protein solubility was 17.6, 15.0 and 19.0% for muscle I, II and III, respectively. Gels were induced upon heating protein suspensions to above 40°C. A major peak in loss modulus ( $G''$ , viscosity) and storage modulus ( $G'$ , elasticity) due to protein denaturation and aggregation was observed within 48-50°C for all samples. A secondary  $G'$  peak around 54°C was evident in samples I and III, but not in sample II.  $G'$  values for samples I and III at the end of heating (72°C) and cooling (25°C) were greater than that for sample II. Muscle I and III contained slightly higher proportions of white fibers and lower percentages of red fibers than muscle II, suggesting that variations in protein functionality among animals of different age groups may be related to fiber type distributions.

## INTRODUCTION

Skeletal muscles are classified into fast-twitch glycolytic (FG), fast-twitch oxidative glycolytic (FOG), and slow-twitch oxidative (SO) groups based on their contraction speed and histochemical characteristics. FG, FOG, and SO muscles are also often referred to as white, intermediate, and red muscles, respectively, according to their color intensity. Animals in different growth stages or physiological maturities have variable distributions in these fiber types. For instance, in growing pigs, the proportion of red fibers in most skeletal muscles increases with age up to 16 wk, while white fiber content decreases steadily (Suzuki and Cassens, 1980). The two major fiber types, FG and SO, differ considerably not only in ultrastructure, but also in chemical composition, including percent fat and protein and content of crosslinked collagen. In particular, myosin and several other myofibrillar proteins exist in various isoforms depending on the fiber types. Recent studies showed that myofibrillar proteins from white and red fibers had distinctly different physicochemical and functional properties important to meat processing, such as solubility, rheology, and gel-forming ability (Xiong, 1994). These functional discrepancies may account for, to some extent, variability of physical attributes in processed muscle foods made from different beef cuts or fiber types. The objective of this study was to compare semimembranosus muscle myofibrillar proteins from cattle at different ages with respect to their solubility and gelation properties in relation to fiber types.

## MATERIALS AND METHODS

**Muscle samples.** Chianina-breed steers were raised on a grain-supplemented diet containing maize silage, wheat straw, soybean meal and sugar beet pulps. Crude protein and cellulose content were 13.2 and 23.0%, respectively. At the ages of 10, 16 and 22 mon, eight animals were slaughtered. Mean live weights of the 10-, 16- and 22-mon cattle at slaughter were 400, 580 and 720 kg, respectively. After storage of the carcasses in a 2°C cooler for 24 h, semimembranosus muscle was excised, immediately frozen at -80°C, and subsequently stored at -29°C for not longer than 2 mon before use.

**Fiber typing.** Frozen muscle was thawed at 4°C overnight. Muscle fiber types, classified as FG, FOG, and SO, were determined using a histochemical staining method described by Nicastrò and Maiorano (1994).

**Myofibrillar proteins.** Myofibrils were isolated from thawed muscle following the procedures described by Xiong and Blanchard (1993), using a buffer containing 0.1 M NaCl, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM NaN<sub>3</sub> (pH 7.0). Myofibril pellets were kept on ice and used within 24 h of isolation.

**Protein solubility.** Myofibrils were suspended (5 mg/mL protein) in 0.6 M NaCl, 50 mM Na<sub>2</sub>HPO<sub>4</sub> (pH 6.0) buffer. After overnight extraction, protein solubility (%) was determined by centrifugation as described (Xiong and Blanchard, 1993).

**Gelation and rheological measurement.** Myofibrillar protein suspensions (20 mg/mL in 0.6 M NaCl, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.0) were subjected to heating (1°C/min) from 20 to 72°C, and then cooling (1°C/min) back to 25°C, in a temperature-programmable thermal device mounted to a Bohlin VOR rheometer. Sol → gel transformation and viscoelastic characteristics of thermally induced protein gels were measured using small strain (0.02) oscillatory testing for shear stress (Xiong and Blanchard, 1993). Shear storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were recorded at 30-s intervals during testing.

## RESULTS AND DISCUSSION

The semimembranosus muscle was comprised mainly of white fibers. At 10 mon of age, 46% white and 22% red fibers were found in the muscle with the remaining 32% being in the intermediate fiber category (Fig. 1). The percentage of red fibers decreased by 50% as the animal age increased from 10 to 22 mon. Apparently, much of the oxidative red fibers was converted to the more glycolytic intermediate fibers. White fiber content exhibited much smaller changes during this maturation process. Solubility of myofibrillar proteins from whole muscle decreased slightly from young beef (10 mon) to mature beef (16 mon), but increased from 16 to 22 mon. There appeared to be a relationship between protein solubility and white and red fiber distributions in the sense that the increase in protein solubility corresponded to the increase in white fiber content and decrease in red fiber content. Our previous work with poultry red and white muscles also indicated greater solubility of proteins from fast-twitch fibers than from slow-twitch fibers (Xiong and Blanchard, 1994). Therefore, the protein solubility increase from 16 to 22 mon probably resulted from an accumulation of glycolytic white fibers and diminishing of oxidative red fibers.

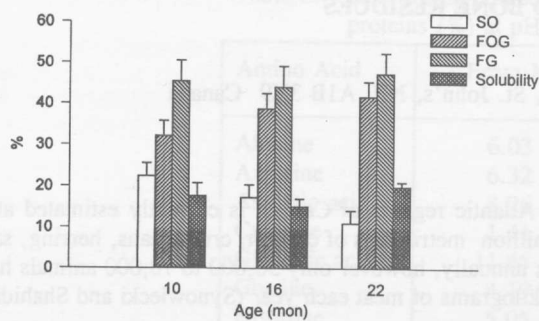


Fig. 1 - Distribution of red (SO), intermediate (FOG) and white (FG) fibers in semimembranosus muscle ( $n = 8$ ), and whole muscle protein solubility ( $n = 3$ ) of 10-, 16-, and 22-mon old cattle.

Upon heating to about 40°C, myofibril suspensions (sols) formed soft, viscoelastic gels. Independently of animal age,  $G'$  increased markedly with temperature from 40 to 50°C, indicative of formation of elastic gel networks (Fig. 2A).  $G''$  also increased abruptly, and this occurred at 38°C (instead of 40°C in  $G'$ ; data not plotted). Because  $G''$  is a measure of viscous responses, the increase in  $G''$  ostensibly resulted from unfolding of proteins. The denatured proteins would have a larger bulk and an enhanced reactivity, enabling protein-protein and protein-water interactions to occur to form crosslinked gel matrices. A major transition in  $G'$  was observed at 50°C on all three muscle protein samples. However, a secondary peak around 54°C was conspicuous only in samples from 10- and 22-mon old cattle.  $G'$  in all three gels plunged abruptly

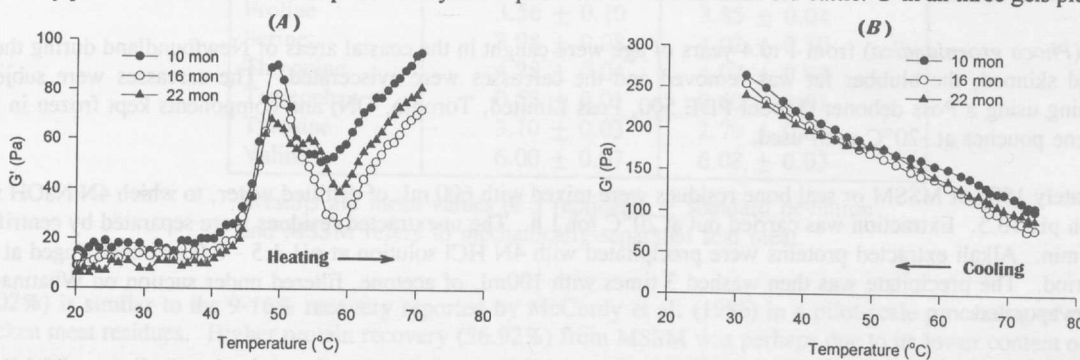


Fig. 2 - Rheograms of myofibrillar proteins revealing changes in storage modulus ( $G'$ ) during sol  $\rightarrow$  gel transformation upon heating (A) and during cooling (B). ( $n = 4$ ).

to a minimum, at 57°C for 10-mon old cattle, and 59°C for 10- and 16-mon old animals, which can be attributed to denaturation of light meromyosin and rearrangement of inter-peptide bonds. Based on a study on chicken muscle actomyosin, Wang and Smith (1995) ascribed the  $G'$  drop also to denaturation of actin and actin-myosin interactions.  $G'$  increased again after 60°C, and the ascending rheological curves of the three gels essentially paralleled. However, the final  $G'$  values (at 72°C) of 10- and 22-mon animal protein gels were greater than that of 16-mon animal protein gel. Presumably, the disparities in gel characteristics between the three animal age groups were due to variations in muscle fiber types. When cooked gels were cooled,  $G'$  increased and the increase was inversely proportional to the temperature (Fig. 2B), probably due to reinforcement of hydrogen bonds. Cooling tended to diminish differences in elastic deformation characteristics of the three gels. Therefore, discrepancies among the three age groups in heat-induced gelation were likely a manifestation of kinetic variations. Our postulation is that proteins from animals of different ages followed slightly different reaction pathways during the formation of gel networks. However, once an equilibrium was established, these gels may possess similar network architectures, thus, exhibiting similar viscoelastic responses when external forces are exerted on them.

## CONCLUSIONS

Gelation of bovine semimembranosus myofibrillar proteins is a dynamic process, revealing complex viscoelasticity changes during heating. Animal age affects myofibrillar protein extraction and solubility, its gelling behavior, and gel characteristics probably due, in part, to different distributions of fast and slow fibers. However, there is no direct or simple relationship between animal age or fiber types and protein functionality, suggesting that other muscle indigenous factors may be involved in regulating the functional performance of myofibrillar proteins.

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