EFFECTS OF PROTEIN CONCENTRATION, PH, IONIC STRENGTH ON HEAT-INDUCED GELATION PROPERTIES OF MYOSIN FROM RABBIT SKELETAL MUSCLES

X. L. Xu, G. H. Zhou, H. B. Huang

Key laboratory of Agricultural and Animal Products Processing and Quality Control, Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095 P.R China

Background

The textural quality of reconstructed and comminuted meat depends on the functional properties of muscle proteins, especially the gelling, binding, emulsification of extracted proteins and water holding capacity (WHC) of the meat product (Boyer *et al* 1996b). Heat-induced gelation is the result of the nature and denatured muscle protein interactions, including inter-protein hydrogen, ionic linkage, hydrophobic interaction and so on. However, the equilibrium among protein-protein, protein-solvent and elements gravitation-repulsion are the most important factors in forming the fine three-dimensional gel matrix. Myosin accounts for about one-third of total muscle protein, 50%~55% of myofibril protein, morever, it has a lot of functional properties in determining the textural quality of meat products. Among these functional properties, wonderful gelling capacity of myosin has been aroused much attention from meat scientists (Siegel *et al* 1979, Boyer *et al* 1996a). Previous literatures indicated that a lot of factors such as protein concentration, pH, ionic strength, type of animal and muscle, extracting process of myosin, heating pattern, non-meat additives had significant effects on heat-induced gelation properties.

Objectives

The purpose of this study was to find out the effects of protein concentration, pH, ionic strength on the hardness, WHC and ultrastructure of heat-induced gelation of myosin from rabbit (New Jersey White rabbit) skeletal muscles (*Psoas major*, PM and *Semimenbranosus proprius*, SMp).

Materials and methods

Male rabbits with liveweight 2~3kg were obtained from Jiangsu Academy of Agriculture Science. After being fed with water only for about 18h, the rabbits were slaughtered and the PM and SMp muscles were used for extracting myosins. The extracting process referred to method of Nauss *et al* (1969), Hermansson *et al* (1986) and Wang *et al* (1994) with a little modification. Myosin concentration was measured by Biuret method (Gornall *et al* 1949) and the composition of each fraction was analyzed by SDS-PAGE on 10% slab gels according to Jun-yao Guo(2001). Myosin (15mg/ml) was held in 7ml plastic centrifugal tube (tubes were previously weighed as W_0 and the tube and protein weight was W_1), then was programmed heated from 20ºC to 65ºC at a rate of 1ºC/min and kept at 65ºC for 20min. Then gels were cooled to ambient temperature and held at 4ºC overnight .

Gel **hardness** (g) was measured with TA.XT2i Texture Analyzer (Stable Micro Systems Ltd, England). with P5 probe (5mm DIA CYLINDER STAINLESS). The test mode was TPA fracture.mac, and test speed was 0.5mm/sec. After centrifugation at 10,000×g, 4ºC, supernatant was decanted, the gel and tube was reweighed as W_2. The **WHC(**%) was calculated as $[(W_2-W_0)/(W_1-W_0)]\times100$. Each test was carried out with three repetitions. Duncan's Multiple-range test was used for ANOVA analysis with SAS8.2. For **SEM examination**, samples were prepared as described by Boyer *et al* (1996b) and observed with a 200 Hitachi Scanning Electron Microscopy (SEM, Hitachi Co Ltd, Japan) at an accelerating voltage of 20kv.

Results and discussion

Both the gel hardness and WHC linearly increased with the gradual increase of protein concentration (Figure 1A), and the differences were significant ($p<0.05$). Gels had maximum hardness of 15.18g for PM myosin , and 14.87g for SMp myosin at 15mmol/l. The maximum WHC was 66.85% for PM myosin and 51.89% for SMp myosin respectively. There was no difference in hardness between two types of myosin gels ($p>0.05$), however, when the myosin concentration was above 8mmol/l, PM myosin gels had higher WHC than SMp samples($p<0.05$).

pH played a significant role in the matrix formation and water retention of the myosin gels. Both the hardness and WHC varied with the increase of pH from 5.0 to 8.0 (Figure 1B). PM myosin gel had the highest hardness (20.79g) at pH6.0, while SMp myosin had a peak at pH5.5 (29.39g). WHC increased from pH 5.0, and reached maximum at pH6.0, and then decreased at higher pH. At the peak point, WHC was 82.99% for PM myosin gel which was higher than that of SMp myosin (67.27%)($p<0.05$).

Gel hardness decreased sharply when ionic strength increased from 0.2 to 0.6($p<0.05$) and decrased slowly at higher ionic strength (0.6~1.0) (Figure 1C). In gels at ionic strength 0.2, maximal hardness reached 45.60g and 44.61g for PM and SMp myosin respectively. In contrast, WHC decreased when ionic strength ranged from 0.2 to 0.4, and then increased gradually until ionic strength reached 1.0. PM myosin gel had maximal WHC (79.87%) at ionic strength 1.0, with no difference with that(74.56%) at ionic strength 0.2 ($p>0.05$). The similar profile in WHC occured for SMp myosin gel, which was 78.51% at ionic strength 1.0 compared to 76.69% at ionic stength 0.2. At all points, there was no difference between these two myosin gels.

Gel ultrastructure could be obtained by SEM only when myosin concentration was higher than 5mg/ml. PM myosin formed a looser network at concentration 5mg/ml than at 10,15 mg/ml (Figure 2B, Figure 3C). When heated, myosin molecules aggregated into cross-linkage with diameter 0.1~0.2μm(Figure 2A). The ultrastructure was a bit disorganized and pore size varied greatly from 0.2 to 1.3μm. Gel with 10mg/ml had a homogeneous granular structure exhibiting spherical corpuscles (diameter 0.1~0.2μm),uniform cavities(diameter~0.3μm), but the cross-linkages were shorter compared to those in 5mg/ml gel, which indicated more matrix formed in the course of heating.

Myosin formed a homogeneous network at 0.6mol/l KCl with spherical corpuscles exposed to the surface, short cross linkages, small and regular (diameter~0.3μm) cavities. But when the myosin was equilibrated in 0.2mol/l KCl by overnight dialysis (4□), another entirely different three dimension matrix formed and gel hardness and WHC increased significantly ($p<0.01$) compared to those at 0.6 mol/l KCl (Figure 1C). Gels at 0.2mol/l KCl formed a strand-type network (Figure 3-A, B, D, E). And PM gel was more homogeneous than SMp gel. Diameter of the strands is less than 0.1μm, and the length varied from 0.5 to 2.5μm. Most of the holes in PM gels were near circinal (most with diameter0.2~0.5μm). In some way, SMp myosin gave rise to denser strand than PM myosin. The strands tended to array in the same direction, so a kind of zonary or sandwich-like structure occured with small hole being in the strand region.

Ultrastructure of myosin gels at pH5.0 and pH5.5 showed serious protein-protein and protein-solvent interactions based on the charge distribution in myosin molecules(Figure4). When heated (pH5.0), molecules denatured and aggregated. Myosin conglomerations developed into column-like cross-linkages (diameter0.15~0.3μm, length 0.8~1.5μm). At pH5.0, the ultrastructure seemed to result from a "collapse" with long (0.6~1.2μm) and thin(~0.1μm) strand. Large cavities were left in these networks. Myosin formed consistently distributed three-dimension ultrastructure at pH6.0 and pH6.5 respectively. While molecules at pH6.5 aggregated into denser structure than pH6.0, a small sheet-like aggregate evenly distributed throughout the gel matrix. When pH increased to pH7.0, pH7.5 and pH8.0, gel structure became coarser with larger granular and holes in the network. In coincidence, the repulsion between molecules increased and coarser structure came into being as a result of more negative ionic in the protein.

Many studies have pointed out the difference between myosin gels of different muscle type, and generally concluded that white muscles (fast-twitch, like PM muscle) exhibited a higher gel-forming ability than those from red muscles (slow-twitch, like SMp muscle). (Egelandsdal *et al* 1995, Culioli *et al* 1993, Xiong, Y.L.1994). This, on the whole, resulted from the different cross-linking capacity of myosin heavy chain, and also the difference of surface hydrophobicity of denatured myosin and temperature discrepancy at which myosin denatured. The result of this experiment conducted was not the same with those represented in other literatures.

Salt and pH could affect the water balance and charge equilibrium in muscle and muscle product. Offer and Knight (1988) suggested that this balance was determined by (a) electrostatic repulsion, (b) restraining forces in structural protein, (c) chemical potential in the system. Water can be held in the gel by capillarity, electrostatic interaction at the protein surface and among the network. It has been known that myosin can only be soluble at high enough ionic strength (>0.3), but water holding capacity was not the same as the number of soluble protein in the solution. Heat-induced gelation could still have good WHC at ionic strength 0.2(Figure 1C). As demonstrated earlier (Boyer *et al* 1996a), myosin formed strand-type gel at ionic strength 0.2 and short cross-linkage gel with aspheric granular on the surface at ionic strength 0.6. Although there were large cavities in strand-type network, the thick strands were necessary for higher hardness and still could hold much water in the strand strips (Figure3A, B, C, D), While the granular structure lost much water when gel was under centrifugation at 10,000×g, 4ºC. Feng and Hultin(2001) also found that strong gels with

good WHC could be formed under low ionic strength conditions, and suggested that electrostatic repulsion of proteins is a major driving force behind gel formation and WHC.
Water loss decreased when pH increased from 5.0 to 6.0,and then increased above pH6.0, indicating that the iso-electricity point might be near pH6.0. This was the same as Kristinsson *et al* (2003). Morita, *et al* (1987) also found that PM muscle protein formed the hardest gel at pH6.0, while SMp gel was hardest at pH5.5. But at high ionic strength (0.6mol/l KCl) and high pH (>6.0), gel hardness and WHC were lower than at other conditions, which could result from electric screening behind this phenomenon, weakening the repulsion between neighboring matrix charges.

Conclusions

Myosin solution could form hard enough heat-induced gel with acceptable WHC only when concentration was above 5mg/ml. At ionic strength 0.2, gel had higher hardness and good WHC with strand-type ultrastructure, while myosin at ionic strength 0.6 aggregated into homogeneous network with aspheric granular on the surface and short cross-linkage throughout the gel matrix. Strong gel and good WHC could be formed at pH5.5 or pH6.0 at ionic strength 0.6. When pH raised above 6.5, myosin denatured and aggregated into coarse network. Gel hardness decreased with increase of ionic strength, but the maximal WHC was obtained at ionic strength 1.0. There was little difference between gelation properties of PM and SMp muscle myosin.

References

1 Boyer, C., Joandel,S.,Roussilhes, V.,Culioli, J. and Ouali, A.1996a.Heat-induced gelation of myofibrillar proteins and myosin from Fast- and Slow-Twitch rabbit muscles. J Food Sci. 61,6:1138-1142.

2 Boyer, C., Joandel, S., Ouali, A. and Culioli, J.1996b. Ionic strength effects on heat-induced gelation of myofibrils and myosin from fast- and slow-twitch rabbit muscles. J Food Sci. 61,6: 1143-1148.

3 Culioli,J., Boyer,C.,Vignon,X.and Ouali,A.1993.Heat-induced gelation properties of myosin: Influence of purification and muscle type. Sci. Aliments. 13:249-260.

4 Egelandsdal,B.,Martinsen,B.and Autio,K.1995.Rheological parameters as predictors of protein functionality-A model study using myofibrils of different fiber-type composition. Meat Sci.39: 97-111.

5 Feng, Y.,Hultin, HO.2001.Effect of pH on the rheological and structural properties of gels of water-washed chicken breast muscle at physiological ionic strength. J. Agric. Food Chem. 49:3927-3935.

6 Gornall, A.G., Bardawill, C.J. and David, M. M.1949.Determination of serum proteins by means of Biuret raction.J.Biol.Chem.177: 751-766.

7 Jun-yao, Guo.2001.Handbood of protein electrophoresis. Chinese Science Press. 86.

8 Hermansson, A. M., Harbitz, O., Langton, M. 1986. Formation of two types of gels from bovine myosin. J. Sci. Food .Agric. 37:69-84.

9 Kristinsson, H.G.and Hultin, H.O.2003. Role of pH and Ionic strength on water Relationships in Washed Minced Chicken-breast Muscle Gels. J. Food Sci. 68:917-922.

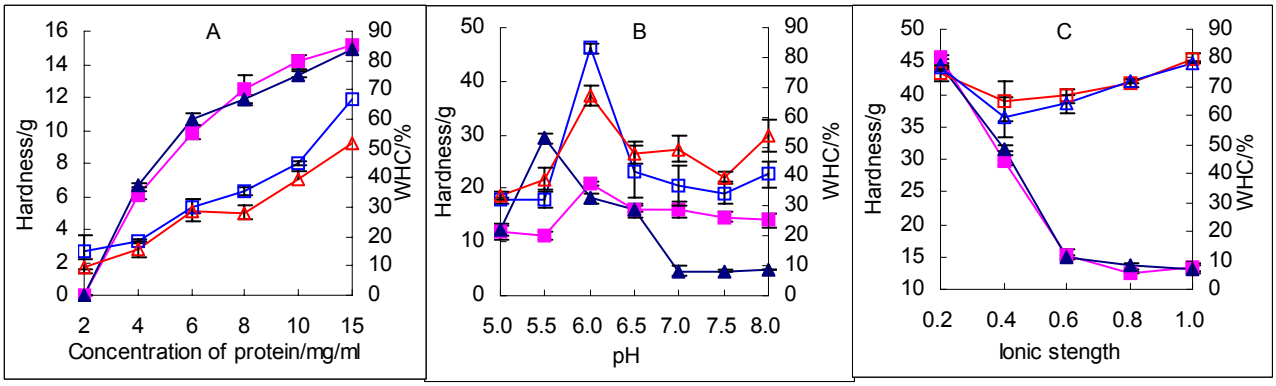
10 Morita, Jun-Ichiro., Choe, II-Shin., Yamamoto, K., Samejima, K. and Yasui, T.1987. Heat-induced gelation from leg and breast muscles of chicken. Agric Biol Chem. 51,11: 2895-2900

11 Nauss, K. M., Kitagawa, S., Gergely, J.1969. Pyrophosphate binding to and adenosine triphosphatase activity of myosin and its proteolytic fragments. J Biol Chem.244, 3: 755-765

12 Offer, G., Knight, P. 1988, The basis of water holding in meat. Part1: General principles and water uptake in meat processing. In: Lawrie R. editor. Developments in meat science.London.UK: Elsevier Applied Science Publishers. 63-72

13 Wang, S. F., Smith, D. M. 1994, Dynamic Rheological Properties and Secondary Structure of Chicken Breast Myosin As Influenced by Isothermal Heating. J. Agric. Food Chem.42: 1434-1439

14 Xiong, Y.L.1994.Myofibrillar protein from different muscle fiber type: Implications of biochemical and functional properties in meat processing. Crit.Rev.Food Sci.Nutr.34:293-320



Hardness: ■-PM ▲-SMp WHC: □-PM △-SMp

Figure 1 Effect of concentration (A), pH (B) and ionic strength(C) on the gel hardness and WHC of myosin

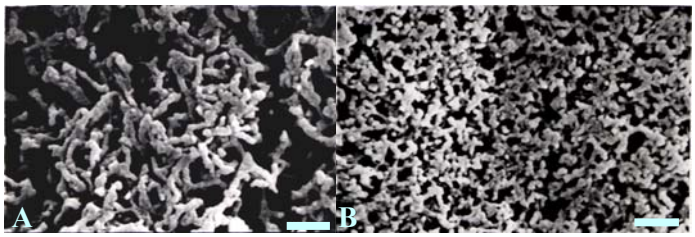


Figure2 Scanning Electron Micrographs of heat-induced PM myosin gels(pH6.0,0.6mol/l KCl) at different concentration PM (A 5mg/ml, B 10mg/ml), Bar length is 1 μ m

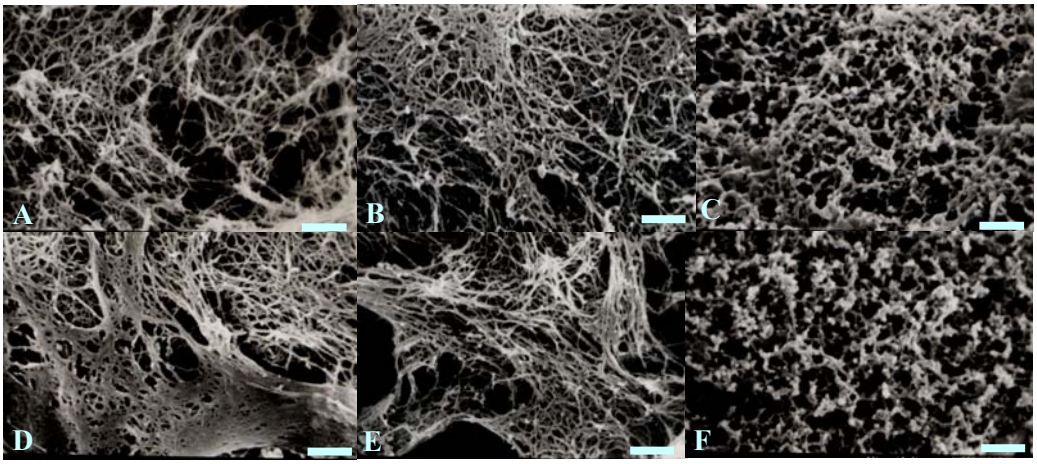


Figure 3 Scanning Electron Micrographs of heat-induced gels of myosin(pH6.0,15mg/ml) at different ionic strength A,B 0.2mol/l KCl PM;C, 0.6mol/l KCl PM; D E 0.2mol/l KCl SMp； F 0.6mol/l KCl SMp. Bar length is 1 μ m

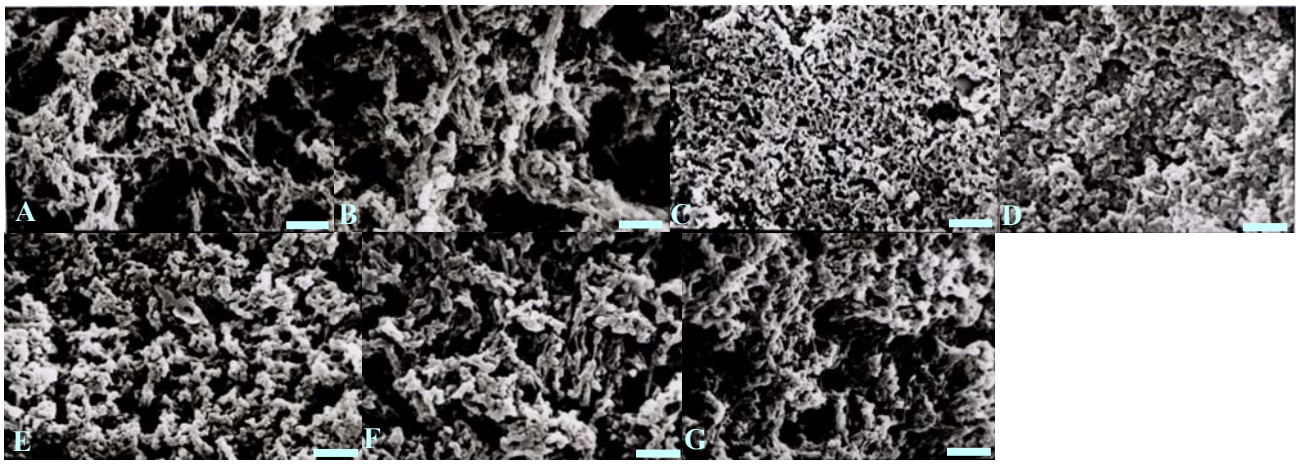


Figure 4 Scanning Electron Micrographs of PM myosin gels(0.6mol/l KCl,15mg/ml) at different pH PM (A pH5.0,B pH5.5,C pH6.0,D pH6.5,E pH7.0,F pH7.5,G pH8.0),Bar length is 1 μ m