4 - 26 RECENT ADVANCES IN THE STUDY OF MYOSIN AND MYOFIBRIL GEL FORMATION

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The pioneering work by Fukazawa and coworkers (1) implicated myosin as the key to binding quality in comminuted meat products. The conclusion to be drawn from a great body of subsequent work is that binding depends exclusively on contractile proteins, extracted from the myofibrils, and their gelation by heat during processing. A comprehensive review on the gelation functionality of muscle proteins in structured meat products will be published shortly (2).

Although pure actin solutions form gels at slightly enhanced temperatures (<40°C) further heating causes the gels to collapse into strings of denatured globules (3,4). Nonetheless, actin displays a synergistic effect: The gels of myosin-actin mixtures, within a certain range of proportions, are stronger than corresponding myosin gels (5), presumably as a result of initial actomyosin formation (3). The crucial role of myosin is indisputable, but binding in meat products constitutes a complex phenomenon involving more than one factor (6). Thus, studies of myofibril gelation have also been undertaken.

MYOSIN GEL FORMATION

Various aspects of myosin/actomyosin gelation upon heating have become more clear through recent, extensive work (7-16). Among the findings reported are: Gelation in 0.4-0.6 M KCl proceeds by initial aggregation of the myosin "heads"

(disulphide bonds form) and subsequent network formation upon unfolding of the tail (rod) portion of the molecule. Gel strength is dependent on pH, having a maximum at pH 6.0 (0.6 M KCl); in the range 0.4-1.0 M (pH 6.0) salt concentration makes no difference. In 0.1-0.2 M KCl, depending on pH, interfilamental aggregation of myosin heads on filament surfaces are responsible for gel formation and gel strength is positively correlated with salt concentration.

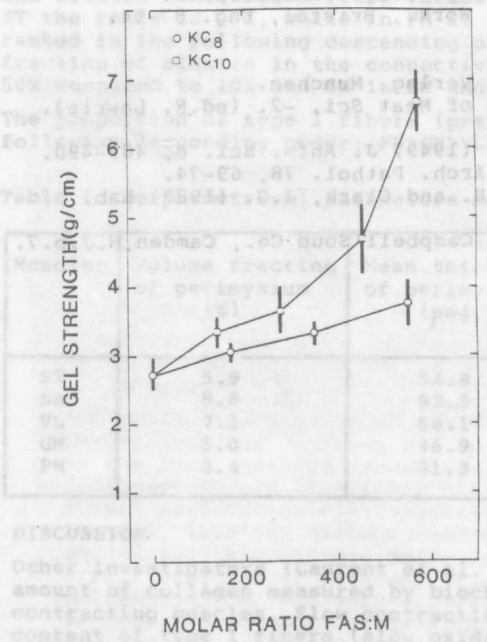


Fig. 1: Gel strength (by penetrometry) of myosin (M) gels as a function of the amount and type (chain length) of fatty acid salt (FAS) present; 9 mg prot./ml, pH 6.0, 0.6 M KCl. Bars indicate ± S.E.M.

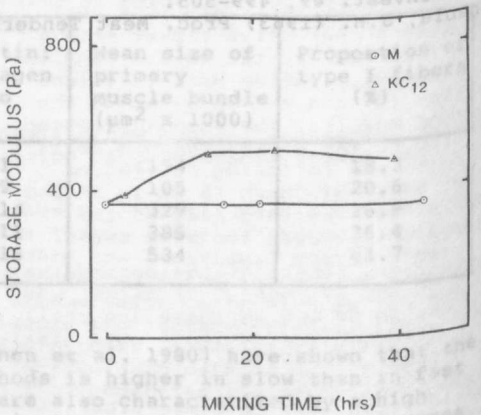


Fig 2: Storage modulus (gel strength by oscillating shear) of myosin gels, with (KC_{12}) and without (M) potassium laurate in a molar ratio of 250, as a function of interaction time at 5°C prior to gelation by heating; 10 mg prot./ml, pH 6.0, 0.6 M KCl.

Effect of lipids: Clearly, comminuted meat batters represent a far more complex system than the pure protein solutions/suspensions referred to in the preceding paragraph. One difference is that lipids are present in the batters. We are investigating interactions of selected lipids with myosin, and initial findings have revealed marked effects of some fatty acids/fatty acid anions on myosin gelation. As seen in Figure 1, presence of caprylic acid (C_8) (added as the potassium salt) is of no significance in the concentrations tested; capric acid (C_{10}), on the other hand, displayed a pronounced effect on gel formation (interaction period: 2 hrs at 5°C prior to gel formation by heating). Lauric acid (C_{12}) required longer interaction times (Figure 2), but the maximal effect, obtained after about 12 hrs, was

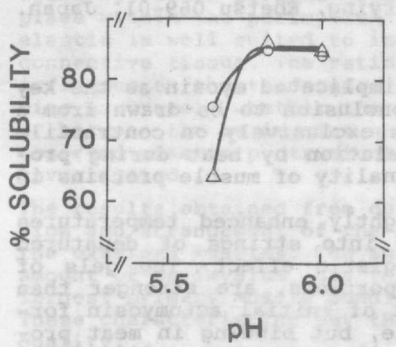


Fig. 3: Relative solubility of myosins as a function of pH; 10 mg prot./ml, 0.6 M NaCl.

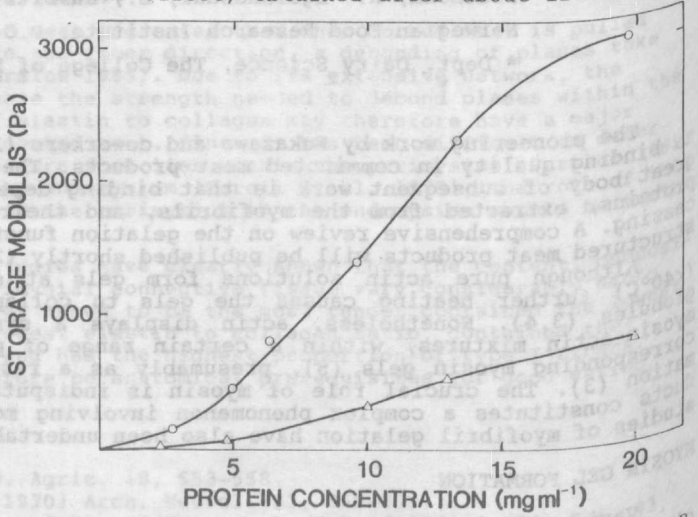


Fig. 4: Storage modulus of myosin gels as a function of protein concentration; pH 6.0, 0.2 M KCl.
○ white myosin (M. cutaneus trunci)
△ red myosin (M. masseter)

larger than the one recorded for the same molar concentration of the C_{10}-acid in Figure 1. (A complete report will appear in J. Food Sci. (17)). Fatty acids with longer alifatic chains would be of greater practical interest to meat scientists, but their higher temperatures of transition, into a solubilized state, excluded them from these experiments.

Effect of muscle fiber type: Another aspect of applied meat science pertains to the well-known fact that meats from different muscles display different binding properties when used as alternative constituents in meat batters. Undoubtedly, one reason for these differences rests with the difference in relative proportions of red (slow, type I) and white (fast, type II) muscle fibers. For example, Maesso *et al.* (18) pointed out that broiler's breast meat had superior binding quality compared to leg meat. Investigations of white-myosin and red-myosin from appropriate broiler muscles revealed that white-myosin gels always exhibited greater gel strength than did red-myosin gels (19).

We have studied the gel formation of white-myosin and red-myosin from bovine muscles, *M. cutaneus trunci* and *M. masseter*, respectively. The difference in properties between the two types of myosin is observed already as different solubilities at low pH, Figure 3. Gel strength (Figure 4) was found to differ even more than reported for broiler myosins (19): In 0.2 M NaCl white-myosin gels were roughly four times stronger than the red-myosin gels. In 0.6 M NaCl the difference was smaller but, nonetheless, quite distinct during the entire gelation process (Figure 5). It should be pointed out that our bovine myosin gels displayed a different dependence on pH (Figure 6) compared to those of avian myosin gels (19). Present work in our laboratory is aimed at gaining an understanding of the physical chemistry behind these differences.

MYOFBRIL GEL FORMATION

The most striking difference between myosin and myofibril gelation, respectively, is that myofibrils yield weaker gels. Figure 7 shows that, under identical conditions of total protein concentration, pH and ionic strength, pure myosin gels are almost 3 times as strong as the corresponding myofibril gels. Undoubtedly, the primary reason for this difference rests with the lower concentration of myosin in the myofibril suspension prior to heat treatment. We have found our myosin preparations to give gels whose strength (storage modulus) is an exponential function of the protein concentration, the exponent being 1.8-2.0. Salt (sodium or potassium

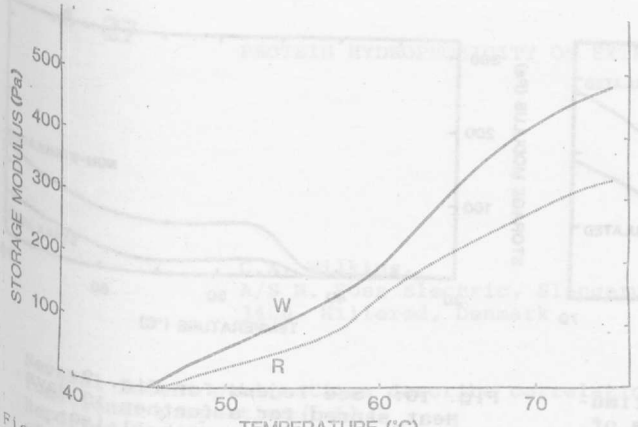


Fig. 5: Thermograms of the storage modulus of myosin gels; 10 mg prot./ml, ph 6.0, 0.6 M NaCl. W: white myosin; R: red myosin, cf. Fig. 4.

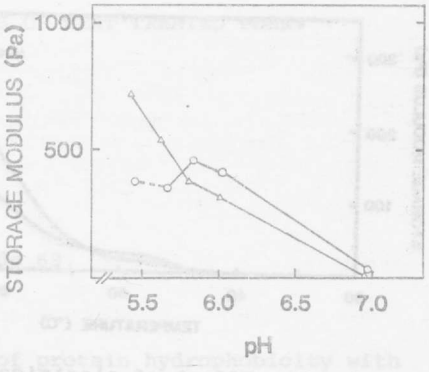


Fig. 6: Storage modulus of myosin gels as a function of pH; 10 mg prot./ml, 0.6 M NaCl. Symbols as in Fig. 4.

chloride) is well known to aid in the solubilization of myosin, and, as expected, it is seen in Figure 7 that lower salt concentrations result in even weaker myofibril gels. If myofibril gel strength is investigated as a function of protein extractability from the myofibrils, the curve in Figure 8 is obtained. The levelling off of the curve at the higher values may be related to extraction of proteins other than myosin. However, the conditions during gelation also varied (the higher the ionic strength, the higher the extractability) and must be expected to have had an effect.

Effect of electrical stimulation: Whether electrical stimulation of beef carcasses has effects of practical importance beyond preventing cold shortening has remained a question of dispute. We have investigated the effects of electrical stimulation at the myofibrillar level. Myofibrils were isolated from bovine *M. longissimus dorsi*; carcass halves served as the source, one half being stimulated, the other

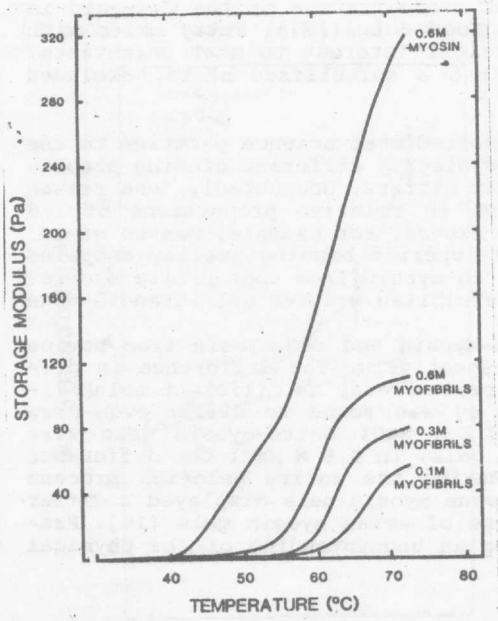
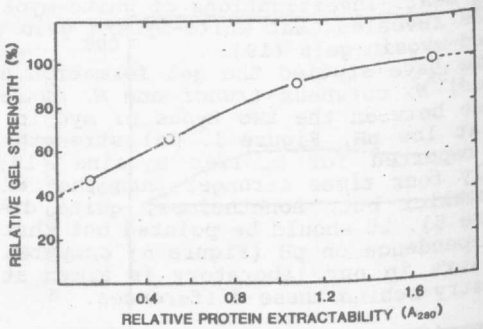


Fig. 7: Thermograms of the storage modulus of myosin gels (0.6 M NaCl) and of myofibril gels (0.6, 0.3, and 0.1 M NaCl); total prot. conc.: 10 mg/ml; pH 6.0.

Fig. 8: Relative gel strength of myofibril gels as a function of the relative protein extractability of the myofibrils; total prot. conc.: 10 mg/ml; pH 6.0.



not. Figure 9 appears to leave little doubt that stimulation causes myofibrils to yield weaker gels. As seen from Figure 10, this effect is even more pronounced when the meat is stored for two weeks prior to myofibril isolation. We are not yet in a position to explain the mechanisms behind the observed differences. Our studies of protein extractability have shown, however, that electrical stimulation is of no consequence for this characteristic of the myofibrils.

As is evident from the above, our understanding of meat proteins and their interactions with other food constituents is still incomplete in terms of allowing scientifically based prediction and control of binding properties and texture formation.

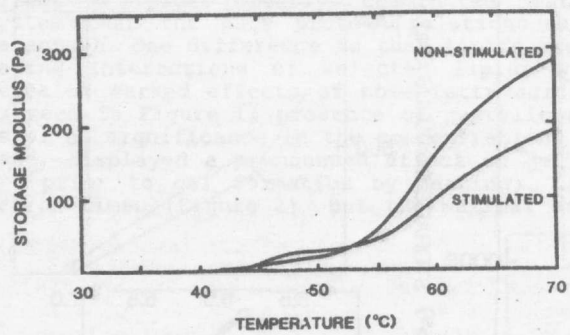


Fig. 9: Effect of electrical stimulation on the storage modulus of myofibril gels; total prot. conc.: 10 mg/ml; phosphate buffer, pH 6.0, containing 0.6 M NaCl, 5 mM pyrophosphate and 5 mM $MgCl_2$. Meat stored at 11°C overnight prior to myofibril isolation and thermal gelation.

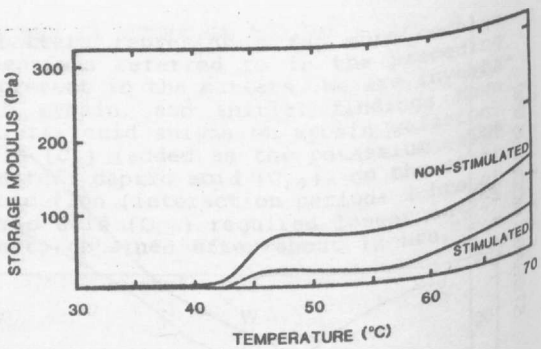


Fig. 10: See legend for Fig. 9. Meat stored for a further 13 days at 4°C prior to myofibril isolation and thermal gelation.

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