# THERMALLY-INDUCED CONFORMATIONAL CHANGES OF NATURAL ACTOMYOSIN EXTRACTED FROM NORMAL AND PSE PORK *LONGISSIMUS*

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**Introduction**

Thermal unfolding and gelation properties of meat proteins, including myosin, salt soluble protein and myofibrillar protein with different species, pH and ionic strength have been intensively studied. Myosin plays a very important role in the gelation process in meat and meat products. Actin is important in reinforcing the gel structure of myosin (Yasui *et al.*, 1982). Myosin and actin are present as an actomyosin complex in full and post rigor meat. Natural actomyosin (NAM) can be extracted under mild conditions and is thought to represent the native state of proteins in post mortem pork meat. We have previously (Rathgeber *et al.*, 2002) reported that pale, soft, and exudative (PSE) pork produced meat gels with lower strain values compared to those from normal pork. The cause of poor gelation may be related to denaturation and degradation of myofibrillar proteins, but the biochemical and physicochemical properties of proteins from normal and PSE pork during heating and their gelation mechanisms are not fully understood.

The objective of the present study was to investigate the gelation behavior of NAM extracted from normal and PSE pork. Protein conformational changes during heating were studied by examining alterations of tertiary and/or quaternary structures (surface hydrophobicity and surface sulfhydryl and disulfide contents), while protein unfolding was monitored by determining alterations of secondary structures (α–helical content).

**Materials and Methods**

Pork loins from seven PSE and five normal pigs were selected based on pH at 2 and 24 h post mortem and on amount of drip loss (Normal: $pH_2 > 5.8$, $pH_u < 5.8$, drip loss < 5%; PSE: $pH_2 < 5.8$, $pH_u < 5.8$, drip loss > 5%). Meat was frozen at 72 h postmortem and later used for extraction of pork NAM as described by Wang *et al.* (2005). Heat treatment of NAM involved dilution of extracted NAM to 5.0 mg protein/mL in 6 M KCl, 20 mM $KH_2PO_4/K_2HPO_4$ buffer, pH 7.0 and then heating from 25 to 90°C at a constant rate of 1.3°C/min. An unheated solution kept at 4°C served as a control.

The α–helical content was estimated as described by Wang *et al.* (2005). Protein surface hydrophobicity was determined with the fluorescent probe 1-anilinonaphthalene-8-sulfonic acid (ANS) according to Li-Chan *et al.*, (1985). Total and surface sulfhydryl (S-SH) (also called reactive sulfhydryl) contents of heat-treated NAM were determined in the presence and absence of 8 M urea, respectively, by colorimetric assay using Ellman's reagent 5, 5'-dithio-bis (2-nitrobenzoic acid). The sulfhydryl plus disulfide (SH+SS) content of thermally-treated NAM was measured by using 2-nitro-5-thiosulfobenzoate according to Damodaran (1985) with slight modifications as described by Wang *et al.*, (2003). Disulfide content was calculated as the difference between (SS+SH) and total sulfhydryl contents.

Dynamic rheological properties of NAM (20 mg/mL in 0.6 M KCl, 20 mM $KH_2PO_4/K_2HPO_4$ buffer, pH 7.0) from PSE and normal pork were measured by using an AR-1000 advanced rheometer (TA Instruments, New Castle, DE, USA), which was equipped with a parallel plate measuring system (40 mm diameter). The sample was heated at a rate of 1 °C/min from 20 to 80°C, using a programmable temperature-controlled water bath.

**Results and Discussion**

The α–helical content of NAM from both normal and PSE pork decreased to about 3% upon heating above 70°C, indicating that approximately 90% of the α–helical structure of the actomyosin molecules were unfolded at 70°C and higher temperatures (Figure 1A). The decline of α–helical content of NAM from PSE meat occurred at a lower temperature than that of NAM from normal meat (~54 °C versus 57°C).

The surface hydrophobicity ($S_0$-ANS) of NAM from PSE meat was higher than that from normal meat below 40°C, indicating greater exposure of hydrophobic groups on the surface (Figure 1B). This result was in agreement with our previous report for pork stored at –20°C for up to 6 months (Wang *et al.*, 2005). The $S_0$-ANS of NAM from both meat groups rapidly increased at temperatures above 40°C and reached maximum values at 80°C. Interestingly, $S_0$-ANS of NAM from normal pork was higher than that from PSE meat at temperatures above 55°C, showing greater changes with heating in hydrophobicity of NAM from meat of normal quality. Surface hydrophobicities of NAM from normal and PSE pork at 70°C were 3.6 and 2.4 times greater than that at 40°C.

Possibly, the lower change in development of hydrophobicity of PSE meat would contribute to less structure development during heating.

Concentrations of free sulfhydryl groups declined (disulfide content increased, Figure 1C) during heat treatment of pork proteins, but quantity varied with meat quality. About 44% of available sulfhydryl groups formed disulfide bonds or other permanent chemical bonds at 80°C in NAM from normal, while this number was only 26% for thermally-treated NAM from PSE pork.

Dynamic thermal rheology of NAM from normal and PSE pork during heating from 25 to 85°C gave very similar rheological patterns. However, there were a few subtle differences. Namely, changes of G' of NAM from PSE pork always occurred at a lower temperature during thermal treatment compared with NAM from normal pork (Figure 1D). The temperature at the lowest storage modulus (G') during thermal rheology was in accordance with that at maximum α–helical content decline of NAM. In addition, the NAM from normal meat had a slightly higher initial G' and G", and δ at peak than corresponding values for NAM from PSE pork. Although the NAM from normal and PSE pork seemed to have different gelation kinetics during heating from 50-60°C, their final elastic response was similar.

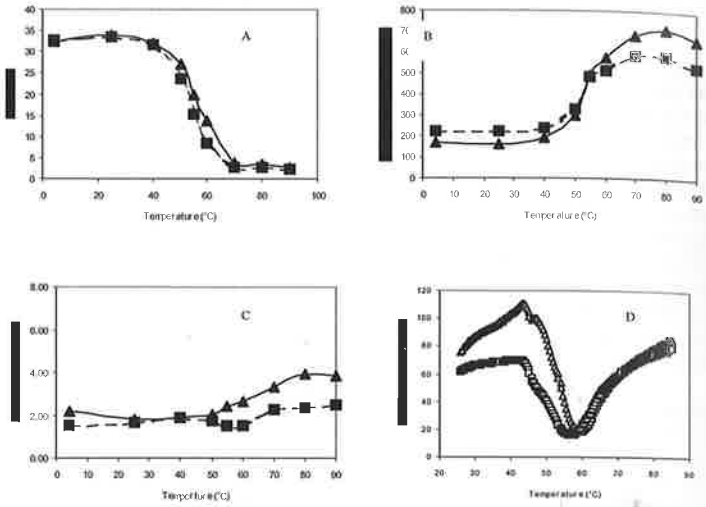


**Figure 1:** Alpha-helical content (A), surface hydrophobicity (B), disulfide content (moles per $10^5$ g of protein) (C) and storage modulus (D) of NAM from normal (▲) and PSE (■) pork during heating. Heating rate was 1°C/min for storage modulus (dynamic rheological profile); 1.3°C/min for all other parameters.

**Conclusions**

Overall, NAM from normal pork underwent aggregation with a higher extent of hydrophobic interaction and disulfide bonds, as well as higher temperatures at maximum velocity for conformational change and unfolding than that from PSE pork. As a consequence, NAM from normal pork had superior rheological properties.

**References**


Damodaran, S. (1985). Estimation of disulfide bonds using 2-nitro-5-thiosulfobenzoic acid: Limitations. *Analytical Biochemistry, 145,* 200-204.

Li-Chan, E., Nakai, S., and Wood, D. F. (1985). Relationship between functional (fat, heating, emulsifying) and physicochemical properties of muscle proteins. Effects of heating, freezing, pH and species. *Journal of Food Science, 50,* 1034-1038.

Rathgeber, B. M., Boles, J. A., Pato M, and Shand, P. J. (2002). Myosin degradation and impaired gelation observed in PSE pork. In *48th International Congress of Meat Science and Technology* (pp. 590-591). Volume II.

Wang, H., Liceaga-Gesualdo, A., and Li-Chan, E. C. Y. (2003). Biochemical and physicochemical characteristics of muscle and natural actomyosin isolated from young Atlantic salmon (*Salmo Salar*) fillets stored at 0 and 4°C. *Journal of Food Science, 68,* 784-789.

Wang, H., Pato, M. D., and Shand, P. J. (2005). Biochemical properties of natural actomyosin extracted from normal and pale, soft and exudative pork loin after frozen storage. *Journal of Food Science, 70,* C313-320.

Yasui, T., Ishioroshi, M., and Samejima, K. (1982). Effect of actomyosin on heat induced gelation of myosin. *Agricultural and Biological Chemistry, 46,* 1049-1059.