Symposium: Meat Science and Muscle Biology: Postmortem Changes in Myofibrillar Protein and the Associated Contribution to Meat Quality

556 Historical perspective of postmortem changes in myofibrillar proteins and their relationship to meat quality. F. C. Parrish*, *Iowa State University*, *Ames*.

Myofibrillar proteins make up the largest amount of muscle protein and they are largely responsible for postmortem changes in rigor mortis, tenderness and water holding capacity. The changes of myofibrillar proteins and the mechanism of these changes in the tenderization process have received much attention. Proteolysis has long been thought of as the mechanism of postmortem tenderization. The definitive proof of proteolysis and its relationship to tenderness, however, had eluded scientists until studies were initiated in the late 1960's. A series of innovative studies on the changes in molecular properties of myofibrillar proteins in postmortem muscle led to the discovery of Z disk degradation and myofibril fragmentation by a calcium activated endogenous protease. The protease was subsequently named calcium activated factor (CAF). Further extensive studies found CAF had a high requiring calcium form, m calpain, and a low calcium requiring form, μ calpain, and an inhibitor, calpastatin. Other studies on CAF showed myofibril fragmentation and the simultaneous disappearance of troponin T and appearance of a 30 kDa component were strongly related to beef steak tenderness. The 30 kDa component was only in tender beef steaks. The term "myofibril fragmentation tenderness" was introduced to describe a state of beef tenderness. The degradation of titin and nebulin in postmortem beef muscle was found to be due to CAF. Also, degradation of titin, nebulin, and troponin T was greater and more rapid in a tender than in a tough beef muscle. Titin was more rapidly degraded in muscle from steers, than it was in muscle from bulls and mature cows, too. Degradation of specific structural proteins (titin, nebulin, filamin, desmin, and troponin T) by μ calpain at low pH and temperature was similar to their degradation in postmortem muscle. Other studies on calpain activity and its inhibitor, calpastatin, and the affects of calcium infusion of carcasses and their relationship to tenderization have proven valuable. From these historical studies it can be concluded that limited and specific proteolysis of key structural proteins in the I band of the myofibril are caused by μ calpain and results in postmortem tenderization.

Key Words: Proteolysis, 30 kDa Component, Calpain

557 Calpain biology and postmortem meat tenderization. D. E. Goll*, J. P. Camou, J. A. Marchello, S. W. Novak, and V. F. Thompson, *University of Arizona*, *Tucson*.

It is now widely accepted that the calpains have an important role in the postmortem proteolysis that contributes to tenderization. It remains unclear, however, how the calpains function in postmortem muscle, where the temperature after 6-12 h is near 2-4°C, the pH is near 5.8, and the calpain inhibitor, calpastatin, is present. Studies have suggested that μ-calpain is the primary protease responsible for postmortem proteolysis, but μ-calpain has very little activity after 2-3 d postmortem, even when assayed at pH 7.5 and 25°C. It has been suggested that loss of μ-calpain activity during postmortem storage is an artifact caused by exposure to high ionic strength. We have partly purified calpastatin and both μ- and m-calpain from 11-d postmortem bovine muscle without exposing them to ionic strengths above 200 mM during purification. Both calpains are nearly inactive after 11 d postmortem when assayed at pH 7.5 and 25°C; the inactive μ-calpain can be detected with antibodies. Western analysis shows that postmortem μ-calpain consists largely of 76- and 28-kDa subunits, so the small subunit of μ-calpain is not autolyzed to any appreciable extent during postmortem storage. Based on results of *in vitro* assays, this 28/76-kDa molecule would be expected to be proteolytically active, and it is unclear why it has no activity. *In vitro* assays done at 25°C and using purified calpains show that at-death m-calpain is nearly inactive proteolytically at pH 5.8 but that at-death μ-calpain has some activity at pH 5.8. Zymogram assays, however, show that 1-d postmortem μ-calpain is nearly inactive at pH 5.8, even when assayed at 25°C. After partial purification, calpastatin activity is detected in 11-d postmortem muscle, but this activity is less than the calpastatin activity in at-death muscle. Postmortem calpastatin polypeptides are smaller than 70-kDa and seem less effective (higher K_i values) than at-death calpastatin in inhibiting the calpains. The results thus far suggest that postmortem calpastatin may not be an effective calpain inhibitor, but they raise a number of questions about whether the calpains have any appreciable proteolytic activity after 2-3 days of postmortem storage.

Key Words: Calpain, Tenderness, Postmortem

558 Relationship of postmortem changes in myofibrillar protein to meat quality. E. Huff-Lonergan* and S. Lonergan, *Iowa State University*, *Ames*.

Variations in tenderness and water-holding capacity are major quality problems that significantly affect the profitability of the meat industry. It is clear that early postmortem biochemical and biophysical processes, including proteolysis, contribute to the development of water-holding capacity of pork and tenderness of both pork and beef. For decades, it has been known that tenderness of meat is improved by holding the product at refrigerated temperatures for periods of time ranging from 1-4 weeks postmortem. This process is referred to as postmortem aging or conditioning. During the aging period, major structural changes in the tissue occur. Many of these changes are associated with myofibrils and their linkages to the sarcolemma. Since myofibrils make up nearly 80% of the volume of the muscle cell, their disruption will greatly influence meat tenderness. Even prior to 24 hours postmortem, significant proteolysis of proteins linking myofibrils to the sarcolemma and to each other occurs. Other changes correlated with increased tenderness include breakages within the myofibrils leading to their increased fragility and fragmentation. The increase in myofibrillar fragmentation is indicative of the amount of proteolysis and subsequent tenderization that has taken place. Many proteins involved in meat quality are substrates of the protease μ-calpain. Microenvironmental factors such as pH and ionic strength and oxidative conditions influence the ability of calpain to degrade myofibrillar substrates. Both μ-calpain and m-calpain have slower rates of activity against myofibrillar protein substrates at pH values and ionic strengths similar to those found in postmortem muscle. Alterations in pH and/or ionic strengths may cause conformational changes that allow an increase in the hydrophobicity and aggregation of the enzyme. Likewise, pH/ionic strength changes may alter the conformation of substrate proteins and render them less susceptible to

cleavage by μ-calpain. In addition, post-translational modifictactions of the calpains and their myofibrillar substrates may also contribute to the development of meat quality attributes.

Key Words: Muscle, Meat Quality, Postmortem Changes

559 New methods to investigate changes in meat and myofibrillar proteins. E. Veiseth*, *Matforsk*, *Ås, Norway*.

Meat tenderness improves during postmortem cooler storage largely due to calpain-mediated proteolysis of key myofibrillar and associated proteins. This proteolysis does not appear to be random, and several studies have been undertaken to determine which specific proteins are degraded and what structural changes are associated with tenderisation. While most structural changes described until now occur at the ultrastructural level (e.g. fracturing of myofibrils), we have focused on changes occurring at the microstructural level (i.e. fracturing of muscle fibres and their detachment from connective tissue).
Our objective was to investigate relationships between microstructural changes, calpastatin activity, and Warner-Bratzler shear force (WBSF) in aged Longissimus dorsi (LD) muscle from fifty Norwegian Red bulls. The LD muscle was conditioned at 12°C for 10h, and then aged for 7 days at 4°C. Samples for calpastatin measurements were extracted between 24 and 31 hrs postmortem using a heated calpastatin procedure. After 7d, WBSF measurements and microstructural analyses (optical microscopy observations of plastic sections stained with toluidine blue) were performed. Before correlation analyses (SAS, 1999), the data were corrected for the fixed effects of age, batch, and pen within batch. The analysis showed a positive correlation (0.62; $P<0.001$) between calpastatin and WBSF, while negative correlations were found between muscle fibre fractures and both WBSF (-0.33; $P=0.02$) and calpastatin (-0.55; $P<0.01$). These results indicate that muscle fibre fractures resulting from calpain-mediated proteolysis is a major microstructural change responsible for variation in WBSF of bovine LD muscle, while muscle fibre detachments from connective tissue have limited impact.
We have recently begun to investigate changes in muscle structural proteins using proteomics. A combination of protein fractionation, two-dimensional gel electrophoresis, image analysis, and mass spectrometry is used to quantify and identify changes in these proteins during postmortem storage of beef LD muscle.

Key Words: Muscle, Microstructure, Proteomics

560 Post harvest processes that influence myofibrillar protein degradation and meat quality. M. N. Lund[1], R. Lametsch*[1], M. S. Hviid[2], and L. H. Skibsted[1], [1]*University of Copenhagen*, *Frederiksberg, Denmark*, [2]*Danish Meat Research Institute*, *Roskilde, Denmark*.

Modified atmosphere retail packaging with a high level of oxygen (70-80%) finds an increasing use for fresh meat as high oxygen concentrations preserve the bright red colour of fresh meat and increase shelf-life by reducing microbial growth. However, packaging of fresh meat in high-oxygen atmospheres increases oxidation of meat lipids and proteins leading to undesirable off-flavour formation and reduced meat tenderness. Oxidation of meat lipids has been studied extensively during the last decades, while protein oxidation in fresh meat has not received much attention until recently. Oxidation of proteins includes formation of reactive radicals, amino acid derivatives, and cross-linked protein derivatives, which may cause changes in the functional properties like loss of protein solubility and enzyme activity.
Myosin, which constitutes approx. 50% of the myofibrillar proteins, is very susceptible to *in vitro* oxidation of both its cysteine and tyrosine residues and possibly also other amino acid residues (1). We found that *in vitro* myosin oxidation causes formation of long-lived tyrosyl radicals and intermolecular disulfide and dityrosine cross-links which result in polymerization and aggregation of the protein (1). In fresh meat stored in high-oxygen atmospheres, myosin was found mainly to cross-link through intermolecular disulfide formation, which was consistent with a reduction in meat tenderness. In comparison, storage of meat without oxygen resulted in more tender meat and no observable myosin cross-linking (2). Furthermore, we found that addition of antioxidants capable of inhibiting lipid oxidation like a rosemary extract containing phenolic compounds did not inhibit protein oxidation in meat (3).
It is concluded, that protein oxidation of key myofibrillar proteins is a major consequence for meat quality. Notably, the protection of proteins from oxidation by using antioxidants needs further attention.
(1) Lund, MN *et al.* Biochem. J. 2008, doi:10.1042/BJ20071107
(2) Lund, MN *et al.* Meat Sci. 2007, 77, 295-303
(3) Lund, MN *et al.* Meat Sci. 2007, 76, 226-233

Key Words: Protein Oxidation, Tenderness, Myosin Cross-Linking