Application of proteomics in the study of pig meat quality

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• Introduction:

The pig (Sus scrofa) is one of the most important animal species used for meat production. During the genomic revolution of the past decades, the production of pigs and its processing was fundamentally based on new technologies, one of them proteomics. Proteomics is an important cornerstone in functional genome characterization, and like all other functional genomics tools, the aim of proteome studies is to translate genome information into useful biological insight, that will allow scientists to build better hypotheses, with the ultimate goal to find better solutions to challenges in meat science.

The aim of this review is to provide a current status of how proteomics have been used to search and characterize bio-markers related to tenderness of meat quality.

• Material and Methods:

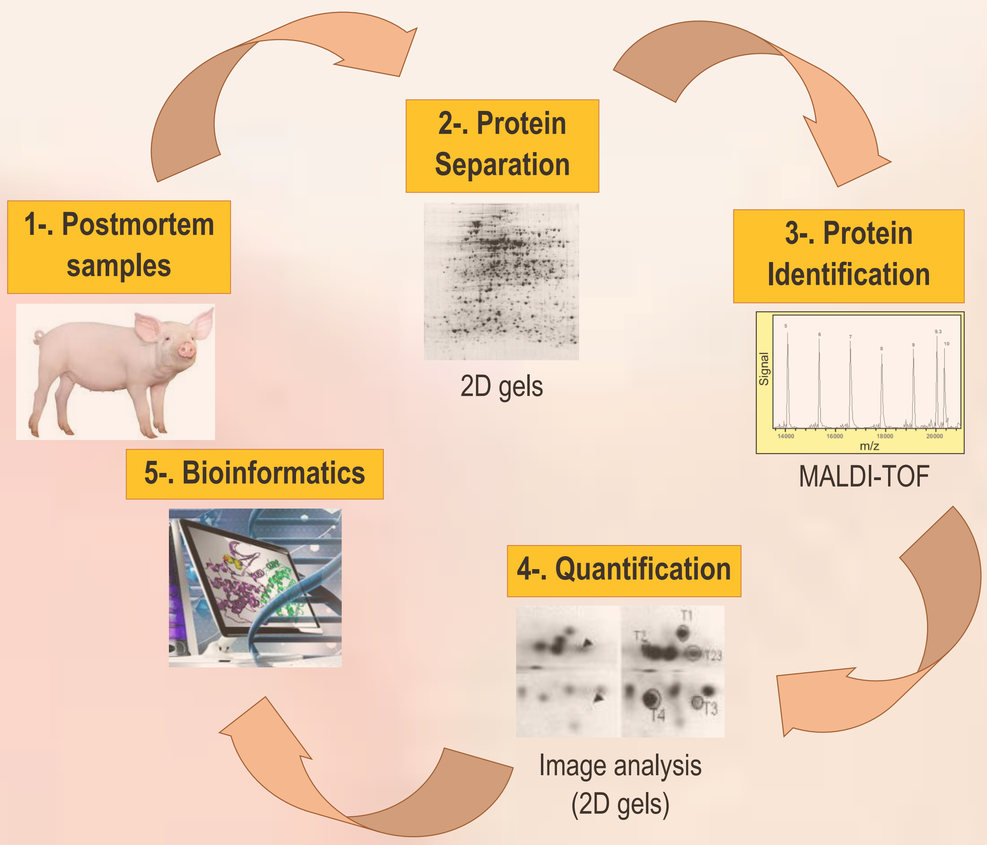

Figure 1. Diagram of methods in comparative proteomics

• Results:

One of the most relevant and innovative studies about the relation between changes in postmortem proteome of porcine muscle and tenderness development area of proteomics studies in pork, was made by the group of E. Bendixen.

Identification of the postmortem proteome changes was based on a comparison of muscle samples taken immediately from animals after slaughter and 72h postslaughter. A total of 345 individual spots were matched and compared, of which 103 spots were found to have changed significantly (P<0,01) during the first 72h. The most notable changes were selected for protein identification by MALDI-TOF MS. Twenty-seven altered spots were identified, and figure 4, shows a representative 2DE display where the migration patterns and the changes in spot intensities are illustrated for the 27 identified proteins. The results clearly show that both actin and myosin heavy chain are the major structural proteins degraded postmortem.

Another study from the same group found that actin and myosin heavy chain were substrates of μ-calpain. It was rather surprising, as it has previously been reported that these proteins were resistant to μ-calpain degradation. However, both actin and myosin heavy chain are poor substrates compared with desmin, as we can see in figure 5.

During the last few years, proteomics has also been applied to investigate proteome changes induced by different pre-slaughter conditions. One example involves a study of compensatory growth in pigs, which has been associated with more tender meat. In a proteome study at slaughter, 7 proteins were changed according to compensatory growth. Among them several stress proteins and glycolytic proteins were decreased in abundance. In the same study 8 proteins were affected 48h after slaughter including both structural proteins and enzymes.

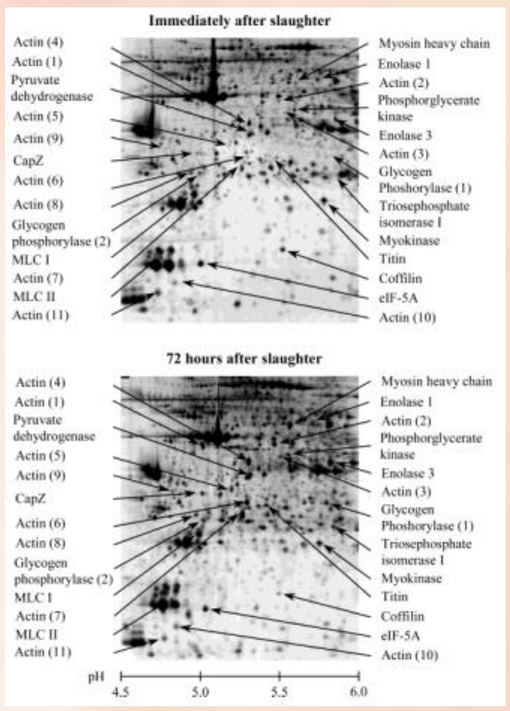

Figure 4. 2DE gels of pig longimus dorsi immediately after slaughter and 72h after slaughter. Arrows show the identified protein in postmortem changes. *J. Agric. Food Chem., 2003, Vol. 51, No. 24, 6992-97*

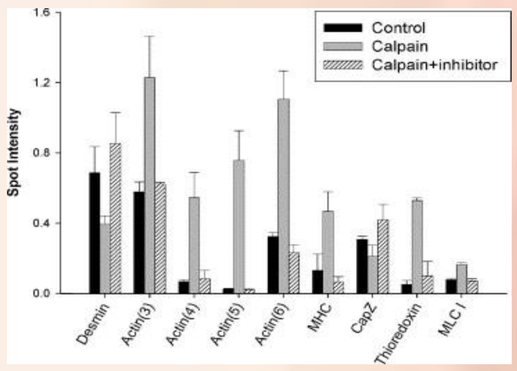

Figure 5. The average spot intensity on the 2DE gels of the identified μ-calpain substrates. The myofibrils were incubated for 4 days with μ-calpain, control without μ-calpain (control), and control with μ-calpain and inhibitor. The difference in spot intensity between the control and μ-calpain was significant (p<0,05). *Meat Science, 2004, 68, 515-521*

• References:

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• Conversion of muscle to meat:

As muscle is converted to meat, many changes occur, including:

1. A gradual depletion of available energy.
2. A shift from aerobic metabolism to anaerobic, favoring the production of lactic acid resulting in the pH of the tissue declining from near neutrality to 5.4-5.8.
3. A rise in ionic strength, in part, because of the inability of ATP-dependent Ca^{2+}, K^{+} and Na^{+} pumps to function.
4. An increasing inability of the cell to maintain reducing conditions.

All of these changes can have a profound effect on numerous proteins in the muscle cell, especially on the proteinase system, which is considered to play a significant role in the tenderization that occurs during postmortem aging.

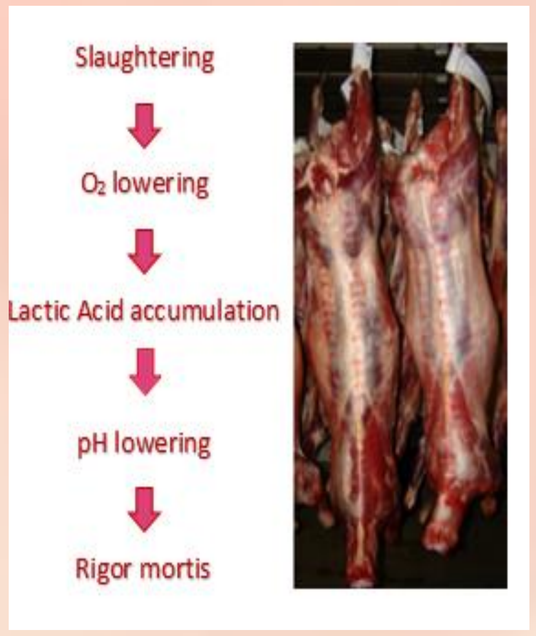


Figure 2. Schematic representation on the use of proteomics in the conversion of muscle to meat..

• Proteinase systems:

- **Calpain:** is a protein belonging to the family of calcium-dependent, non-lysosomal cysteine proteases, expressed ubiquitously in mammals and many other organisms. It is well established that calpain proteases system play a key role in tenderization of meat, and the rate-limiting factor is actually calpastatin-mediated inhibition of postmortem calpain activity.
- **Cathepsins:** were the first lysosomal enzymes that were considered for the study of meat quality. After many studies suggested that there was a sequential action between cathepsins and calpains during meat formation. It was concluded that during early stages of muscle changes were made due to proteolysis by calpains, while delayed changes were due by cathepsin. Finally, meat becomes tender 4 days post-mortem (96 h) and is structurally determined by the separation of sarcomeres.

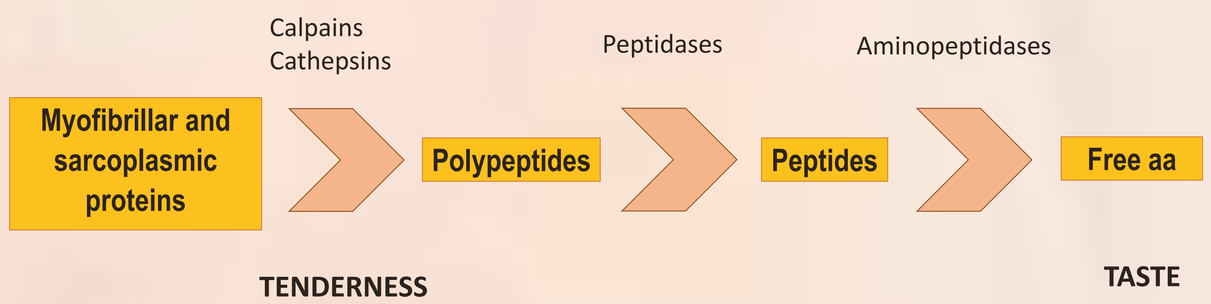


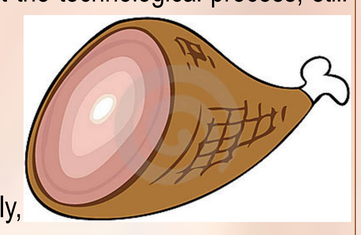
Figure 3. General scheme of proteolysis during the processing of meat.

Protein degradation is not the only process that causes extensive modifications encountered by the pig muscle proteome during the post-mortem storage. These modifications include oxidation and phosphorylation on muscle proteins:

- **Oxidation:** Recent studies have indeed pointed out that protein oxidation is a significant process associated to post-mortem events. In fact, the shift in energy metabolism after slaughter due to the lack of oxygen supply leads to the formation of reactive oxygen species (ROS) that cause oxidative damages to proteins.
- **Phosphorilation:** This modification have also been suggested to play a role in the post-mortem process and hence in meat quality. Two recent studies have reported that in pig meat mainly metabolic enzymes and stress response proteins are heavily phosphorylated post-mortem.

• Cooked Ham :

The industrial production of cooked ham from pork meat involves, as initial steps, the injection of brine and a prolonged meat massage. These processes strongly affect the quality of the final product because they determine the breakage of muscle cells and the release of their protein content. The produced dense exudates act as a glue in the final cooked ham. In order to exploit modern tools to direct the technological process, still mainly based on empirical observations and traditional recipes, we have carried out a comprehensive proteomic analysis of the exudates as a function of brine concentration, temperature, and length of meat massage. Each condition was found to generate specific protein patterns. Peptide mass fingerprinting analysis was applied allowing the identification of proteins, whose presence and/or quantity can be defined as biomarkers of meat processing, and, potentially, of final product quality.



• Conclusion:

1. Meat quality is manifested through a complexity of events in the muscle and their interactions with many environmental stimuli in both the live animal and during the postmortem period.
2. The protease, μ-calpain has the ability to catalyze the degradation of titin, nebulin, filamin, desmin, troponin-T, actin and myosin heavy chain into many of the same degradation products produced in myofibrils from naturally aged meat.
3. There are correlations between the postmortem degradation of actin and myosin heavy chain to tenderness, which clearly indicated that the postmortem degradation of actin and myosin heavy chain influences meat texture.
4. It is concluded that proteomics analysis can be a successful method to identify marker proteins for prediction of meat quality.