Identification of *Post mortem* Protein Degradation of Pork with Two-dimensional Gel Electrophoresis and Mass Spectrometry.

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Background

It is well accepted that meat undergoes a tenderisation process during storage and that proteolysis of key myofibrillar and associated proteins contribute to this process. Several studies have shown that many of the structural proteins such as titin, neubulin, troponin T, desmin, filamin, and vinculin are degraded *post mortem* (Taylor et al. 1995). However, the precise mechanism of the proteolytic activity is still unclear. Two dimensional gel electrophoresis (2DE) is a method that separate proteins according to their individual isoelectric points and molecular weights providing a very high resolution, those making it possible to study quantitative profile of hundreds of proteins *post mortem*. Proteins of interest are identified by using matrix assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS), allowing identification of proteins in the femtomole range.

Objective

The objective of this study was to identify porcine protein changes during the first 48 hours *post mortem*.

Methods

7 littermates originating from a Danish landrace/Yorkshire sow and a Hamshire boar were used in the study. Samples were taken from Longissimus Dorsi immediately after exsanguination and after 4, 8, 24 and 48 hours *post mortem*. The samples were homogenized in a buffer containing 6M UREA, 2M thiourea, 50mM DTT, 1% CHAPS, and 1% carrier ampholytes. The 2DE was performed according to Görg et al. 1995. 50µg protein was loaded onto18 cm IPG-strips covering the pH range 3-9. For separation in the second dimension a 10% SDS-PAGE was used. Proteins were detected by silver staining. The protein pattern of 35 gels were matched and analysed with the BioImage software (Genomic Solution). Proteins of interest were identified as described by Jensen et al. 1998. The proteins were cut out of the gel and digested with Trypsin. The masses of the resulting peptides were detected by MALDI-TOF-MS, and in order to reveal protein identities, peptide mass information was used in database searches.

Results and Discussion

Figure 1 shows a 2DE gel of a sample taken 24 hours after slaughter. It was possible to identify about 1000 spots on the shown gel, illustrating the high resolution of the 2DE gel. In comparison, traditional 1 dimensional SDS-PAGE, only allows separation of 10 to 50 protein bands. By comparing 2DE gels of samples taken at slaughter, 4, 8, 24, and 48 hours *post mortem* it was found that the intensity of several spots changes during the first 48 hours. Twenty of the most notable changes showing a similar profile in all the seven examined animals were cut out and identified (figure 1). The 20 identified spots originate from modification and fragmentation of 11 individual proteins. In figure 2 the quantitative staining profiles for the identified spots are illustrated.

Proteolytic fragments of the structural proteins troponin T, actin and myosin heavy chain increase in intensity *post mortem*, while the full length troponin T is decreasing concomitantly. It was not possible to detect any decrease in intensity of the full-length actin, and by comparing the spot intensities of the actin and myosin heavy chain fragments with the full-length actin it is estimated that only a minor part of actin and myosin is degraded. Several studies have shown troponin T to be degraded *post mortem*, however, both actin and myosin are described as resistant to *post mortem* proteolytic activity (Koohmarie. 1996).

The heat shock proteins HSP27 and αB-crystallin were found to increase *post mortem* reaching a maximum level during the first 4 hours (figure 2). Intensity profiles and information about the molecular weight indicate the increase to be a result of *post mortem* protein expression or modification. This is consistent with previous studies, showing HSP27 and αB-crystallin to be rapidly up regulated during a variety of stress conditions such as heat shock, oxidative stress, and hyperosmotic stress (Welsh and Gaestel. 1997).

15 of the 20 identified spots were fragments of 6 different metabolic proteins. 5 of these are the sarcoplamic proteins glycogen phosphorylase, creatin kinase, phosphopyruvate hydratase, and myokinase, whereas dihydrolipoamid succinyltranseferase is located in the mitochondria.

Conclusion

The results of this study show that the high resolution of 2DE makes it possible to study hundreds of proteins *post mortem* and provide a informative picture of the *post mortem* protein changes. These studies will increase our understanding of the complex process of conversion of muscle to meat. In our future studies, we will study the correlation between *post mortem* protein changes and meat quality parameters.

References

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Figure 1. 2DE gel of sample taken 24 hours *post mortem*. The proteins are separated according to their isoelectric point in the horizontal direction and to their molecular weight in the vertical direction. The arrows show the spots that change *post mortem*

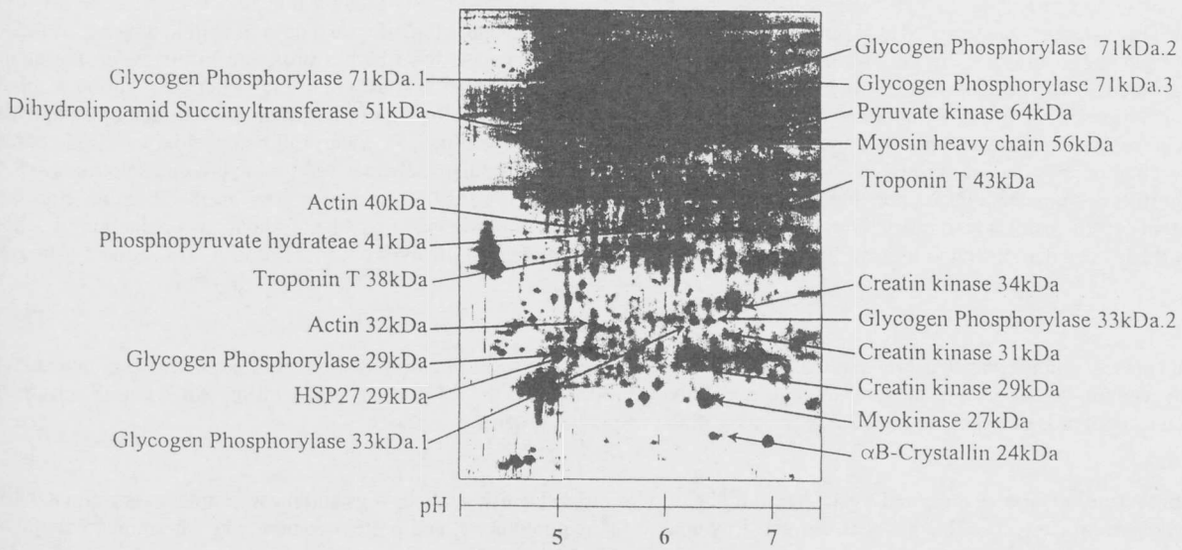

Figure 2. The tables show the staining intensity profile of the identified protein changes. The bars represent the average intensity of the spots based on the observation of seven animals.