PS1.02 'Meatomics' 127.00

Rene Lametsch (1) khd@life.ku.dk
(1)University of Copenhagen, Denmark

Abstract—The use of proteomics in studies related to meat quality ("Meatomics") has clearly shown that this technology has a large potential in the area of meat science. Proteomics has provided a much more detailed picture of the postmortem protein degradation. It has been established that actin and myosin are degraded postmortem and many other muscle protein were found to be degraded that previously not have reported to degraded postmortem. Proteomics has also been used to investigate the post mortem metabolism in muscle and provided further knowledge about the mechanism behind PSE. In future studies will proteomics also be an effective research tool to investigate the relation between meat quality and post mortem protein modifications such as protein oxidation and protein phosphorylation.

R. Lametsch University of Copenhagen, Institute of Food Science, 1958, Frederiksberg C, Denmark. (corresponding author to provide phone: +45 3533 3483; fax: +45 3533 3341; e-mail: rla@life.ku.dk).

Index Terms— protein degradation, protein modification, proteomics.

I. INTRODUCTION

Variation in meat quality traits is a well-known problem. Although extensively researched, the underlying mechanisms of many of the different meat quality traits are not fully understood. Basic knowledge of these mechanisms is essential to reduce the variation in the meat quality traits such as tenderness, water holding capacity and color, and new research tools must be applied in meat science to obtain this knowledge. The large progress in biotechnology in recent years has resulted in the development of new scientific research areas such as genomics and proteomics, which are used to study the complex patterns of gene and protein expression in cells and tissue. The technologies developed within genomics and proteomics have a large potential within food science as gene expression and protein composition of plants and animals have a major impact on the yield and quality of the final food products. Proteomics is the study of the proteome, which is defined as the protein complement expressed by the genome of an organism. The term proteome refers to all the proteins produced by the genome of an organism, like the genome refers to the entire set of genes. However, unlike the genome, the proteome is dynamic and varies with the physiological state of the organism. Because encoded proteins carry out most biological functions, application of proteomics is essential to understand how the organism works. However, the proteome composition also has a major influence on the biophysical characteristics of protein based food products such as meat and several meat quality traits such as tenderness, water holding capacity and color are influenced by the protein composition of the muscle/meat. Hence, proteomics can provide valuable information on the mechanisms influencing the different quality traits to gain a better understanding of these mechanisms. This information can be used to optimize meat production and improve meat quality.

II. TECHNOLOGY IN PROTEOMICS

Proteomics is a very challenging task because of the wide-ranging biochemical heterogeneity of the proteins. As an example, the human genome, which is one of the most extensively studied, contains approximately 20,000 genes, each of which, on average, may produce five or six different mRNAs. Each of these mRNA species are in turn translated into proteins that are processed in various ways, generating in the order of 8-10 different modified forms of each protein. Thus, the human genome may potentially produce on the order of 1.8 million different protein species (1). Furthermore, in muscle cells, myosin heavy chain, actin, titin, and nebulin make up nearly 80% of the total myofibril protein; whereas, other proteins only a present in a few copies resulting in a large dynamic range in the order of 106. An ideal proteomics methodology combines high throughput capability with detection of as many protein products as possible in a sensitive, reproducible and quantifiable manner. The classical method in proteomics is two-dimensional gel electrophoresis (2DE) were the proteins are separated according to their isoelectric point in the first dimension, followed by separation according to their molecular weight in the second

dimension. The proteins of interest are then identified by mass spectroscopy (MS). The combination of 2DE and MS is termed as the gel based proteomics approach. The basic principle of the gel based proteomics approach is that the proteins are separated with 2DE first, then the individual protein are quantified and matched with the use of special software. The proteins of interest are finally identified by the use of MS. The protein detection and quantification are very essential issues in proteomics, as the main purpose of differential proteomics is to study the expression level/amount of proteins. Coomassie blue or silver staining is normally used for protein detection in 2DE. However, they have a limited dynamic range and consequently one can only accurately quantify the subset of proteins that happens to fall within the linear-range region on the 2D gel. In recent years, fluorescent detection of proteins has gained popularity in proteomics research because of a high dynamic range and high sensitivity. Moreover, fluorescent probes that are covalently linked to the proteins before the 2DE analysis have been developed. With this approach, it is possible to make dual and multiple label proteome analyses where the samples are labeled with different fluorescent probes and then mixed together and analyzed on the same 2DE gel (2).

Detection of variation in protein modification is very simple with the use 2DE as one of the separation parameters, the isoelectric point or molecular weight, is altered in the modified form. If the proteins are phoshorylated, the isolectric point will change and the protein will migrate differently during the isoelectic focusing in the first dimension. Furthermore, different types of florescence staining for 2D gels has been developed for the specific detection of protein modifications such as phosphorylation and glycosylation (3). If the proteins are modified by cleavage, the molecular weight of the resulting fragments will change and migrate different in the second dimension. In the subsequent MS analysis of the proteins separated in 2DE, the modification can be characterize and the modification site and type identified (1). It is also possible during the MS analysis to predictable the cleavage site if the protein is degraded (4;5).

Despite the apparent advantages of 2DE for separation of complex protein mixtures, the technique suffers from a number of major drawbacks. The first drawback is linked to the physico-chemical properties used for protein separation, molecular weight and isoelectric point. Proteins with high (> 150 kDa) and low (< 10 kDa) molecular weight and proteins with extreme isoelectric point, in particular basic proteins, are usually not detected in standard 2DE. Another drawback concerns the hydrophobic proteins that are not extracted in the buffers used for sample loading or precipitate during the electrophoretic process. Theses limitations of the 2DE makes it very difficult to investigate many of structural proteins in the muscle because many of them such as myosin heavy chain, titin and nebulin are high molecular weight proteins, with a molecular weight above 150 kDa. 2DE also has some technical limitations. Briefly, the process is time-consuming, labour-intensive and requires significant technical expertise to generate quantitatively and spatially reproducible gels.

These limitations have promoted the development of alternative gel free approaches, mostly using liquid chromatography coupled with MS (LC/MS) for separation, quantification and identification of the proteins. The gel free methods have the advantage over 2DE that they allow examination of high or low abundance proteins in the same analysis and are unbiased with respect to molecular weight, isoelectric point, and hydrophobicity of the proteins. Furthermore, all steps may be automated for high-throughput analysis. The development in LC/MS methods in proteomics is enormous and new and smarter methods are constantly introduced (6).

However, the technological requirement is high and expensive because LC/MS is used the MS using the MS instrument full time during the proteomics experiment and if many samples have to analyzed more than one instrument is needed. Another limitation is that only a few samples are compared in each LC/MS run, normally only two. This makes comparison of multiple samples difficult to undertake. Data sets from different LC/MS runs can be combined after separation analysis, but there is a high likelihood that different sets of peptides will be identified in each LC/MS run.

Finally, the LC/MS methods are not well suited for detection of changes in protein modification as in most LC/MS proteomic methods, only a few peptides from each protein are identified during MS resulting in very low sequence coverage and it is unlikely that changes in protein modification, will be identified. As with protein modification it is unlikely that changes in the proteome caused by protein degradation are detected

with the LC/MS methods. LC/MS methods exist that specifically investigate modified proteins (1) where different enrichment methods are used to isolate proteins wit specific modifications, such as phophorylation or glycosylation. These methods have also been used to quantify changes in the specific modification (7).

III. PROTEOMICS IN MEAT SCIENCE

The quality of raw pig meat is influenced by changes in the muscle/meat proteome caused by different factors such as animal growth, age, rate of glycolysis and post mortem protein degradation. Meat scientists have performed a substantial amount of research on their factors, which has led to considerable quality and compositional improvements. However, the underlying biochemical and physiochemical mechanisms behind the influence of these factors on meat are to some extent still not fully understood. The recent application of proteomics in the field of meat science has provided some interesting and promising results.

Post mortem protein changes in muscle have been investigated with proteomics. (8-10). The studies revealed that a large part of the proteome changes post mortem. The mechanism behind these post mortem changes are to some extent still unclear. However, the main cause of protein changes post mortem is probably protein degradation, as many of the identified changes are protein fragments that increase in spot intensity post mortem (4). But changes in protein modification such as phoshorylation or oxidation that changes the isoelectric point of the proteins or releases from protein complexes most likely also contributes to post mortem protein changes. Even protein expression may to some extent cause some changes post mortem, however, it is unlikely that protein expression causes major changes after the muscle has entered the state of rigor mortis as protein expression is an energy requiring process and the energy is nearly depleted after the muscle has entered the state of rigor mortis (11).

At slaughter the blood supply stops and there is no longer a source of oxygen. This results in a change of the energy metabolism from aerobic oxidative metabolism to anaerobic glycolytic metabolism causing an increase in the formation of lactate and hydrogen ions, which results in a decrease of the pH in the muscle cell. 2DE has been applied to characterize PSE zones in pig muscle and sixteen protein spots were found to be affected by PSE. Myosin light chain 1 (MLC I), fragments of creatine kinase and troponin T were identified as proteins with a higher intensity in PSE meat compared with control meat and it was suggested to be a consequence of a decrease in post mortem proteolysis (12). Another interesting observation was that the two heat shock proteins HSP27 and á-crystallin were absent in the PSE meat (12). Another study showed that the post mortem intensity profiles of HSP27 and á-crystallin in non-PSE meat increased to maximum intensity during the first 4 hours post mortem and remains unchanged in the following period (13). These results indicate that the post mortem change of the two proteins is a result of protein expression or modification. HSP27 and á-crystallin are both believed to participate in the organization and protection of the myofibrils and the expression and modification of these proteins are effected by conditions such as stress. The results obtained on HSP27 and á-crystallin with proteomics analysis indicate that these proteins may affect meat quality through stabilizing the myofibrils post mortem and could also be useful biomarkers for PSE or stress. In another 2DE based proteomics study, the effect of pre-slaughter handling was investigated (10). Two pre-slaughter handling procedures were used, in the first the pigs were transported the day before slaughter and in the other the pigs were transported immediately before slaughter. The intensity of eight spots were significantly affected by the pre-slaughter conditions, two of the spots were identified as F1-ATPase chain B and one as myosin light chain II (MLC II). The intensity of the two F1-ATPase chain B and the MLC II spot were higher in the muscle of the pigs that were transported immediately before slaughter. The reason of the increase in intensity of F1-ATPase chain B is most probably related to accelerated post mortem metabolism. Whereas the increase in intensity of MLC II may be a consequence of changes in phorphorylation of MLC II (10). It has been reported that phosphorylation of MLC affects the Ca2+ sensitivity of muscle contraction (14) and it can be speculated that post mortem changes in the phosphorlytion of MLC may affect meat texture. It is well known that meat tenderizes during post mortem storage, and it is believed that post mortem degradation of the myofibrillar proteins such as desmin, titin and neubulin is the main reason for this improvement in meat tenderness. Proteomics has proved a powerful tool to investigate post mortem protein degradation. More than one hundred protein spots have been found to changes post mortem probably as a consequence of protein

degradation (9;15). Several reports have claimed that neither actin nor myosin heavy chain are degraded post mortem (16-18). However, both Hwang et al. (2005) and Lametsch et al. (2003) found both proteins to be degraded post mortem. Moreover, it was found that some of the actin fragments and the myosin heavy chain fragment correlated significantly with meat tenderness (15;19). Hence, indicating that post mortem actin and myosin heavy chain degradation contribute to meat tenderization. However, it is estimated that only a minor proportion of the á-actin and myosin heavy chain are degraded, as the amount of the actin and myosin heavy chain fragments were much lower compared with the amount of the full-length actin and degradation of full-length actin was not detected. MS analysis of the myosin heavy chain fragment revealed that the fragment is part of the globular myosin (4). It can be speculated that the myosin heavy chain degradation leads to disruption of the myosin/actin interaction which may result in more tender meat. Even if only a minor part of actin is degraded, it is reasonable to believe that it could have an effect on the integrity of the thin filament. Several other structural or structural related proteins, such as myosin light chain, troponin T, desmin, capping protein á1 subunit, cofilin 2, F-actin capping protein, CapZ and titin, were found to degrade post mortem. Many of them have not previously been reported to degrade post mortem (4;8;19).

IV. FUTURE PERSPECTIVES

The relative few studies made of the post mortem changes in meat with proteomics clearly illustrate the large potential for proteomics in meat research. Proteomics has especially proved to be a powerful tool to investigate post mortem protein degradation in meat that will provide valuable information about the complex mechanisms behind post mortem proteolysis in meat. Proteomics can provide information on the cleavage sites and degradation pattern of the proteins degraded post mortem. Furthermore the resulting protein fragments may be used as biomarkers to measure the activity of specific proteolytic enzymes. Such biomarkers could be applied in breeding or to optimize animal slaughter and meat processing to gain more tender meat. Proteomics can also be applied to study post mortem metabolism to provide further knowledge of undesirable meat characteristics such as PSE. Proteomics will also be an effective research tool to investigate the relation between meat quality and post mortem protein modifications such as protein oxidation.

REFERENCES

1. Jensen, O. N. (2004) Current Opinion in Chemical Biology 8, 33-41

2. Tonge, R., Shaw, J., Middleton, B., Rowlinson, R., Rayner, S., Young, J., Pognan, F., Hawkins, E., Currie, I., and Davison, M. (2001) Proteomics 1, 377-396

3. Wu, J., Lenchik, N. J., Pabst, M. J., Solomon, S. S., Shull, J., and Gerling, I. C. (2005) Electrophoresis 26, 225-237

4. Lametsch, R., Roepstorff, P., and Bendixen, E. (2002) Journal of Agricultural and Food Chemistry 50, 5508-5512

5. Larsen, M. R., Larsen, P. M., Fey, S. J., and Roepstorff, P. (2001) Electrophoresis 22, 566-575

6. Pan, S., Aebersold, R., Chen, R., Rush, J., Goodlett, D. R., McIntosh, M. W., Zhang, J., and Brentnall, T. A. (2009) Journal of Proteome Research 8, 787-797

7. Kratchmarova, I., Blagoev, B., Haack-Sorensen, M., Kassem, M., and Mann, M. (2005) Science 308, 1472-1477

8. Hwang, I. H. (2004) Asian-Australasian Journal of Animal Sciences 17, 1296-1302

9. Lametsch, R. and Bendixen, E. (2001) Journal of Agricultural and Food Chemistry 49, 4531-4537

10. Morzel, M., Chambon, C., Hamelin, M., Sante-Lhoutellier, V., Sayd, T., and Monin, G. (2004) Meat Science 67, 689-696

11. Henckel, P., Karlsson, A., Jensen, M. T., Oksbjerg, N., and Petersen, J. S. (2002) Meat Science 62, 145-155

12. Laville, E., Sayd, T., Sante-Lhoutellier, V., Morzel, M., Labas, R., Franck, M., Chambon, C., and Monin, G. (2005) Meat Science 70, 167-172

13. Lametsch, R., Roepstorff, P., and Bendixen, E. (2001) 47th ICOMST, Krakow, Poland 246-247

14. Szczesna, D., Zhao, J., Jones, M., Zhi, G., Stull, J., and Potter, J. D. (2002) J. Appl. Physiol 92, 1661-1670

15. Hwang, I. H., Park, K. S., Kim, S. H., Cho, S. Y., and Lee, K. (2005) Meat Science 69, 79-91

16. Bandman, E. and Zdanis, D. (1988) Meat Science 22, 1-19

17. Huff-Lonergan, E., Mitsuhashi, T., Parrish, F. C. J., and Robson, R. M. (1996) Journal of Animal Science 74, 779-785

18. Koohmaraie, M. (1994) Meat Science 36, 93-104 19. Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., Roepstorff, P., and Bendixen, E. (2003) Journal of Agricultural and Food Chemistry 51, 6992-6997