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Application of proteomics to understand the molecular mechanisms behind meat quality

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Abstract

The proteome is expressed from the genome, influenced by environmental and processing conditions, and can be seen as the molecular link between the genome and the functional quality characteristics of the meat. In contrast to traditional biochemical methods where one protein is studied at a time, several hundred proteins can be studied simultaneously. Proteomics is a promising and powerful tool in meat science and this is reflected by the increasing number of studies emerging in the literature using proteomics as the key tool to unleash the molecular mechanisms behind different genetic backgrounds or processing techniques of meat. Thus understanding the variations and different components of the proteome with regard to a certain meat quality or process parameter will lead to knowledge that can be used in optimising the conversion of muscles to meat. At present, there has been focus on development of techniques and mapping of proteomes according to genotypes and muscle types. In the future, focus should be more towards understanding and finding markers for meat quality traits. This review will focus on the methods used in the published proteome analyses of meat, with emphasis on the challenges related to statistical analysis of proteome data, and on the different topics of meat science that are investigated.
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1. Introduction

In a situation with increased competition with other foods, a predictable meat quality is essential to the meat industry, with tenderness and juiciness being the most important quality attributes as defined by the consumers (Hildrum & Tornberg, 1998; Miller, Huffman, Gilbert, Hamman, & Ramsey, 1995). These traits are influenced both by genetics, environmental factors and processing conditions. However, the underlying molecular mechanisms are far from understood. While the genes remain constant during the lifetime of the animal, the expression of the genes to mRNA and proteins is very dynamic and is regulated by a large number of factors such as environmental and processing conditions. The proteins expressed from the genome may thus be viewed as the mirror image of the gene activity.

The proteome is the protein complement of the genome and consists of the total amount of proteins expressed at a certain time point (Wilkins et al., 1996). In contrast to the genome, the proteome is continuously changing according to factors influencing on either protein synthesis or degradation. Thus, analysing the proteome can be viewed as analysing snap-shots into a system in constant change. In this regard, the proteome can be seen as the molecular link between the genome and the functional quality characteristics of the meat, being expressed from the genome under the given environmental/processing conditions. While the genome contains the information on which genes are

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available, the proteome contains information on which genes are actually being expressed. Thus understanding the variations and different components of the proteome with regard to a certain quality or processing parameter will lead to knowledge that can be used in optimising the conversion of muscles to meat (Bendixen, 2005; Bendixen et al., 2005).

2. Tools for proteomics

Proteomics are the tools used to analyse the proteomes. Over the last decade there have been significant improvements of methods in this field, largely driven by medical science, as a demand for understanding the functions of the genomes following the large genome sequencing projects. While the analytical tools for characterising the genomes have reached a high satisfaction, the corresponding techniques for proteome analyses still need much refinements and developments. Most of the proteomics tools are based on protein separation in at least two dimensions, using either chromatographic methods or electrophoresis, and is usually followed by the use of mass spectrometry (MS). Detailed descriptions of these methods are beyond the scope of this paper and are reviewed elsewhere (Aebersold, 2003; Aebersold & Mann, 2003; Bendixen, 2005; Gorg, Weiss, & Dunn, 2004; Rabilloud, 2002; Reinders, Lewandrowski, Moebius, Wagner, & Sickmann, 2004; Righetti, Castagna, Antonioli, & Boschetti, 2005; Stasyk & Huber, 2004; Steen & Mann, 2004). However, we will focus on the methods for two-dimensional gel electrophoresis (2-DE) as this has so far been the method most widely used in meat science, with special emphasis on the sample preparation and analysis of 2-DE data. A schematic diagram of the work-flow of a proteome analysis is shown in Fig. 1. Usually, the first step is to set up an experiment with different animals, treatments or muscles with different quality traits e.g. colour or tenderness (Step 1). This is followed by protein extraction, 2-DE, image analysis and statistical analysis (Step 2–6). Evaluation of the data and selection of significantly changed proteins are critical steps in the experiments (Step 7–8). Furthermore, identification of the proteins by MS and interpretation of the results (Step 9–10) often lead to new hypotheses and new cycles of proteome analyses to be performed.

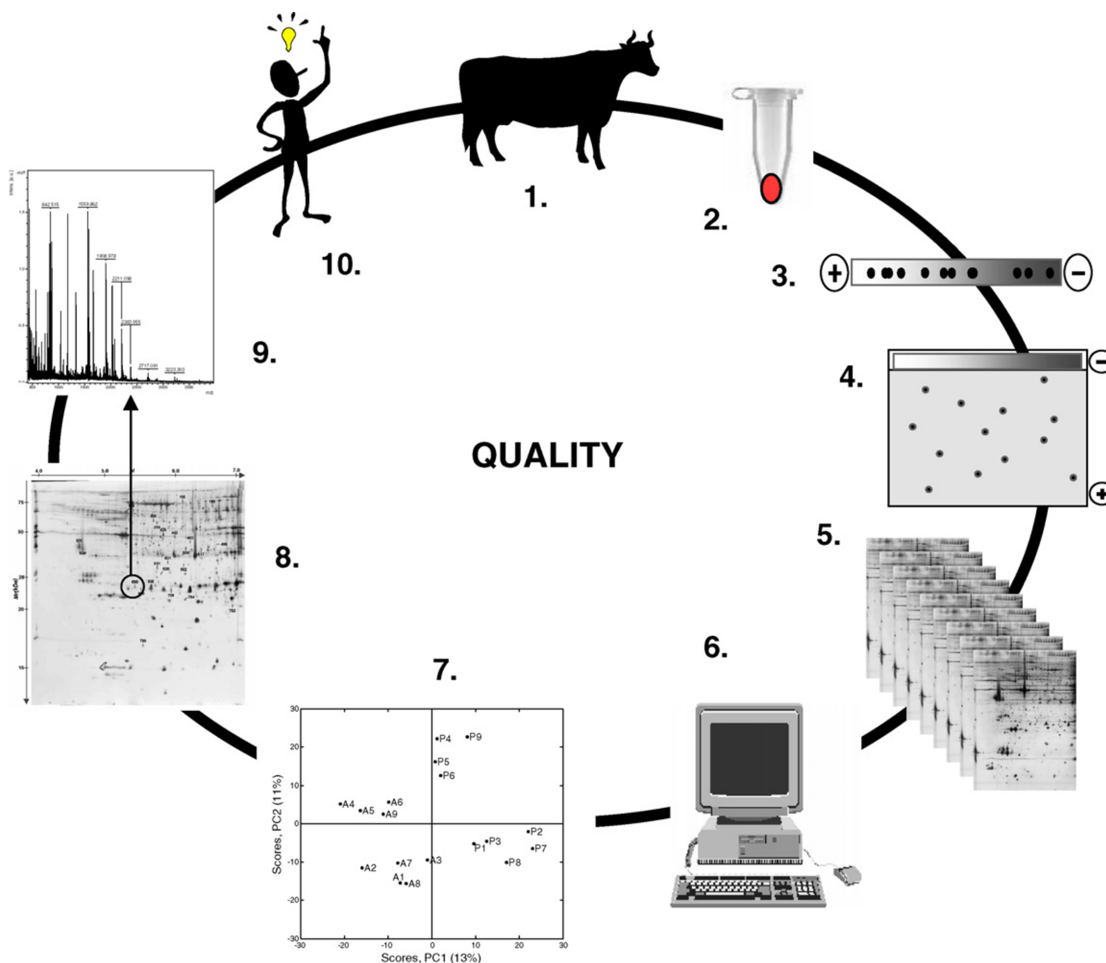

Fig. 1. Schematic drawing of the different steps in the work-flow in proteome analysis using 2-DE and mass spectrometry. (1) Animal or sample chosen for analysis, (2) sample extraction, (3) isoelectric focusing (IEF), (4) SDS-PAGE, 2-dimensional electrophoresis, (5) alignments and comparisons of 2-DE images, (6) data analysis, (7) data interpretation and selection of significantly changed proteins, (8) extraction of significantly changed protein spots, (9) identification of protein spots by MALDI-TOF MS, (10) interpretation of the results.

2.1. Muscle protein fractionation

Several authors have tried to estimate the total number of different proteins expressed from a genome including splice variants and post-translational modifications in a eukaryotic cell, with numbers ranging from 100–500,000 (Righetti et al., 2005; Stasyk & Huber, 2004). These proteins are localised in different compartments. Some are part of large protein aggregates, like the myofibrillar proteins, some are localised in membranes and others are enzymes localised in the cytoplasma. With the help of pre-fractionation of the proteins based on solubilisation and extraction procedures, the enrichment of soluble sarcoplasmic or myofibrillar proteins can be achieved. These proteins will have very different chemical properties which can be used to isolate the proteins in different fractions. Different strategies for fractionation of proteins are reviewed in Righetti et al. (2005). Several issues should be considered before developing a sample preparation strategy. It is advisable to keep sample preparation as simple as possible to avoid protein losses. However, if only a subset of the proteins in the tissue or cells is of interest, pre-fractionation can be employed during sample preparation. Depending on the project and hypothesis different strategies for extraction and pre-fractionation of muscle proteins should be considered. Typically, approximately 1000 different proteins, or 10% of the total number of proteins, can be analysed on one 2-DE image.

The presence of high-abundance proteins in a tissue or cell often masks low-abundance proteins and thus generally prevents their detection and identification in proteome studies. The use of pre-fractionation methods can assist in the identification and detection of low-abundance proteins that may ultimately prove to be informative biomarkers. For comprehensive proteome analysis by 2-DE, pre-fractionation is essential for the following reasons. First, by partitioning the proteome into compartments, the complexity of each compartment is dramatically reduced facilitating spot identification and quantitative analysis. Secondly, there is a pronounced bias inherent in 2-DE towards abundant proteins. This has the effect of masking low-abundance proteins. Pre-fractionation enriches low-abundance proteins. Thirdly, the amount of any given protein that can be resolved on a 2-DE is limited. Pre-fractionation allows the proteins present in a particular fraction to be loaded at high levels, further increasing the representation of low-abundance proteins. Finally, relative to whole cell preparations, the number of proteins that are solubilised during the differential extraction procedures is greatly increased yielding a more comprehensive representation of the proteome.

Several established protein and peptide fractionation techniques include stepwise extractions of proteins, immunodepletion, reverse phase or ion-exchange chromatography and gel filtration (Righetti et al., 2005). The choice of technique is greatly dependent on which subset of proteins that is of interest. In muscle cells, structure proteins, such as actin and tubulin are high abundant proteins and will dominate in 2-DE. Fig. 2 shows the representative 2-DE gels of a bovine LD sample following a sequential fractionation: water-soluble proteins, salt-soluble proteins and remaining proteins. For comparison, a total protein extract from the same animal is also included in the figure.

2.2. Separation by 2-DE

Separation of proteins by 2-DE has been done for decades. However, major technical improvements such as the introduction of immobilized pH gradients have been important for the reproducibility of the method, for a review see (Gorg et al., 2000; Gorg et al., 2004). The information that can be extracted from 2-DE is very informative and provides information on which proteins that are present as well as information on modifications. In short, the proteins are first separated by charge using isoelectric focusing, and then the focused proteins are separated by mass using SDS-PAGE. After separation, different techniques are used to visualise the proteins, resulting in a large number of spots on a 2-DE image of the gel which is subsequently digitalized. It is important to remember that both choice of protein extraction method, pH gradient in the isoelectric focusing step, and staining technology will determine which proteins can be observed and analysed.

2.3. Handling of 2-DE images and statistical analysis of proteome data from 2-DE

Although being very informative, the gel images are complex consisting of a very high number of more or less overlapping protein spots, where the position of the protein spots may vary from one gel image to another. Furthermore, the staining intensity and background may be variable throughout the gels and from one gel to another. Thus, the process of analysing the gel images to search for the information revealed by the proteins is a critical and complex step of the process.

Several commercially available softwares are designed to align and analyse the images from 2-DE experiments. However, due to the noisy appearance of the images this is not an easy task, and improvements are still needed to get an optimal analysis. In principle, there are two different approaches for matching data from one gel to another. One approach is to detect spots in each gel and map the resulting spots from one gel to another. This approach is both time consuming and it faces a number of challenges resulting in a significant number of missing values in the data analysis (Grove, Hollung, Uhlen, Martens, & Faergestad, 2006). An alternative approach is based on alignment of the images instead of matching protein spots. When the images are aligned, spots can be detected across all gels simultaneously using common boundaries around the spots for all gel images (Luhn, Berth, Hecker, & Bernhardt, 2003). Thereafter protein spot tables can be generated without missing values. However, overlapping protein spots and saturated protein spots are still major challenges for the

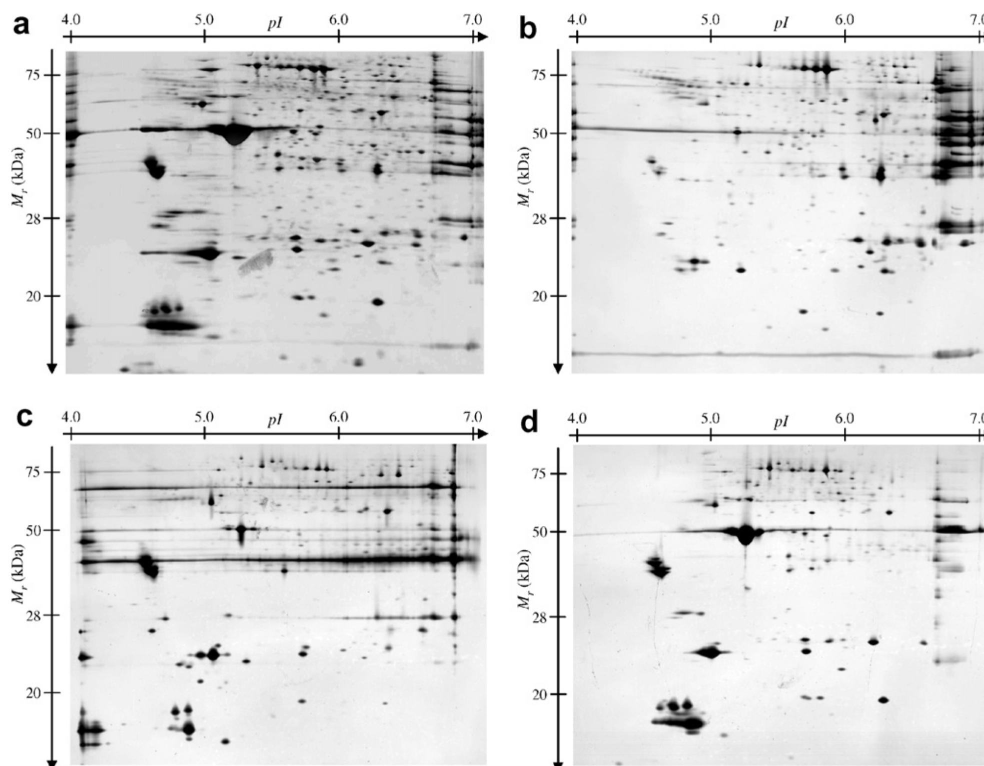

Fig. 2. 2-DE images of proteins from bovine LD muscle separated by pH 4–7 and 12.5% SDS-PAGE. Fifty μg protein was loaded on each gel; (a) total protein extract in urea-buffer (7 M Urea, 2 M Thiourea, 2% CHAPS, 1% DTT, 0.5% IPG 3–10 buffer); (b) proteins soluble in TES-buffer (10 mM Tris, pH 7.6, 1 mM EDTA, 0.25 M Sucrose); (c) remaining proteins from (b) soluble in NaCl-buffer (10 mM Tris, pH 7.6, 0.5 M NaCl); (d) remaining proteins from (c) extracted in urea-buffer, as in (a).

data analysis. A useful approach to overcome these challenges is to analyse the aligned gel images pixel by pixel. Variation from one gel to another even in strongly overlapping protein spots and in saturated protein spots can then be detected.

Proteome data need special attention in analysis and statistical validation of the outcome. In contrast to traditional experiments with few measurements/variables in many samples/animals, the opposite is true for proteomics data. Here the number of samples is usually limiting because of work-load, but the amount of data that is collected from each animal exceeds several hundred observations. This is also a challenge in other –omics data, such as transcriptomics and must be handled with care during the statistical analysis.

Several statistical approaches have been used to analyse proteomics data. Multivariate analyses such as principal component analysis (PCA) (Martens & Martens, 2000; Næs, Isaksson, Fearn, & Davies, 2002) is now included in several softwares for analysis of 2-DE experiments. Multivariate approaches have also been used for selection of significant changes in the 2-DE data according to the design parameters (Jessen, Lametsch, Bendixen, Kjaersgard, & Jorgensen, 2002; Jia et al., 2006; Kjaersgard, Norrelykke, & Jessen, 2006). Assessment of hierarchical clustering methodologies, commonly used in transcriptomics studies, has also been performed on proteomics data (Meunier et al., 2007). The different statistical methods will shed light on different aspects of the proteomics data as has been discussed in several papers (Jacobsen et al., 2007; Maurer, Feldmann, Bromme, & Kalenka, 2005).

2.4. Limitations of proteomics

As with all advanced methods it is necessary to ensure the experimental design is made such that it is possible to analyze the results. Choice of extraction method for the proteins is determining which proteins that can be studied, and proteins that are not extracted will thus not be considered. Very hydrophobic proteins, membrane proteins and high MW proteins are often difficult to solubilise and to analyse by 2-DE (Fey & Larsen, 2001; Gorg et al., 2004). It is also important to keep in mind that whatever method is chosen for proteome analysis there is no protocol yet providing an analysis of the complete proteome in one run. Several groups have tried to compare different strategies on the same samples ending up with partly overlapping results (Bodenmiller, Mueller, Mueller, Domon, & Aebersold, 2007; Frohlich et al., 2006; McDonald et al., 2006). Usually, a few hundred to several thousand proteins can be analysed in one experimental setup, but still this is only a small part of the entire proteome. Thus it is important to draw conclusions based on the proteins that are actually under investigation and not extrapolate the results to the proteins that

failed to be analysed. As discussed in the previous section analysis of the data is not straightforward, and careful consideration should be made to choose the most optimal strategy.

3. Applications of proteomics in meat science

In meat science proteomics is a fairly new tool. However, over the last years several studies are published where proteomics shed light on different aspects of meat, both during the aging process and also in response to different processing conditions. Several species have been studied, such as cattle, pork, lamb and chicken. In most of these studies 2-DE has been used in combination with MS identification of specific proteins as the proteomics tool.

3.1. Proteome mapping

Several proteome studies have been performed aiming at describing the different proteins that are present in a meat sample. This approach can be described as proteome mapping. A proteome mapping study of bovine *semitendinosus* (ST) muscle using a combination of 2-DE and mass spectrometry allowed the detection of roughly 500 reproducible protein spots (Bouley, Chambon, & Picard, 2004). Of these, a total of 129 protein spots corresponding to 75 different gene products were identified by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) MS. Approximately 25% of the identified proteins were involved in metabolic pathways, while 17%, 16%, and 14.5% were involved in cell structure, cell defence, and the contractile apparatus, respectively. However, how these proteins are related to meat quality was not investigated in this study.

A mapping strategy has also been used to compare the proteome expression of sarcoplasmic proteins between four different ovine muscles (Hamelin et al., 2006). Muscle samples were taken within 30 min after slaughter from *longissimus dorsi* (LD), *vastus medialis* (VM), *semimembranosus* (SM), and *tensor fasciae latae* (TL). After 2-DE and data analysis, 77 protein spots were found to be differentially expressed between the muscles, and 47 of these were identified by MALDI-TOF MS. The VM muscle had a different protein expression than the other three muscles. This muscle had the highest proportion of slow-twitch oxidative fibres and the highest abundance of enzymes involved in oxidative metabolism and oxidative stress-related proteins. The TL, on the contrary, had a reduced expression of most of these proteins, which reflects that this muscle had the highest rate of fast-twitch glycolytic fibres.

3.2. Proteome changes due to genetic variations

Genetic variations may cause phenotypic differences that can be studied using proteomics. Changes in expression of bovine skeletal muscle proteins induced by hypertrophy were studied by proteome analyses (Bouley et al., 2005). The muscle hypertrophy was caused by a deletion in the *myostatin* gene, and samples from the ST muscle were taken from bulls that were heterozygote and homozygote for the *myostatin* deletion and from control bulls. The 2-DE analysis allowed the detection of 400 common protein spots, and statistical analyses revealed 28 protein spots that differed between the control and the homozygote bulls, while only one protein differed between the control and the heterozygotic bulls. Changes of proteins in the contractile apparatus and metabolic enzymes indicate that the *myostatin* deletion results in a shift towards a fast-twitch glycolytic muscle type, which is in accordance with the results from the muscle fibre type analyses performed on the same animals. This demonstrates that proteomics reflects the shifts in fibre type in the muscles.

In another study the effect of a quantitative trait loci (QTL) for muscle hypertrophy on sarcoplasmic proteins expression in four ovine muscles was investigated (Hamelin et al., 2006). Thirty minutes after slaughter, samples from LM, VM, SM, and TL were taken for analyses by 2-DE. Only three of the muscles (LM, SM, and TL) were hypertrophied due to the QTL, while the VM was normal. By use of MALDI-TOF MS, 63 spots that were expressed differentially between the genotypes were identified. These changes included increased levels of enzymes involved in the glycolytic metabolism, which indicates a switch towards a more glycolytic muscle type. Moreover, several chaperon proteins were also increased in abundance, which may indicate a requirement for more building blocks to promote myofibril assembly.

3.3. Post-mortem changes

Studying *post-mortem* changes in the proteome will lead to an increased understanding of the biochemical mechanisms behind meat quality traits, such as tenderness. A number of studies have described how *post-mortem* degradation of myofibrillar protein may be involved in meat tenderness, and in particular, the degradation patterns of contractile proteins have been described in detail (Geesink & Koohmaraie, 1999; Hopkins & Taylor, 2002; Melody et al., 2004; Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995).

Lately, proteomics has been used to study changes occurring in muscle during *post-mortem* storage. Total protein extracts from pork LD samples collected at 0, 4, 8, 24 and 48 h after slaughter revealed that 15 proteins were changed, some increasing and some decreasing in abundance after slaughter (Lametsch & Bendixen, 2001; Lametsch, Roepstorff, & Bendixen, 2002). Several of these proteins were identified as fragments of structural proteins such as actin, myosin heavy chain and troponin T. Earlier studies using one-dimensional SDS-PAGE have concluded that actin is not degraded *post-mortem* (Bandman & Zdanis, 1988; Hufflonergan, Parrish, & Robson, 1995; Koohmaraie, 1994). However, using 2-DE the resolution allows for a better separation of the different actin fragments and demonstrates the potential of 2-DE. Some of the

actin fragments observed in *post-mortem* pork muscle was also significantly correlated with tenderness. In addition, several metabolic enzymes involved in energy metabolism were altered in the pork muscle.

During *post-mortem* storage the calpain system is believed to be important for degradation of myofibrillar proteins and development of tenderness (Goll, Taylor, Christiansen, & Thompson, 1991; Koohmaraie, 1994, 1996). In a proteome study in pork LD muscle several of the myofibrillar substrates for μ-calpain were identified (Lametsch, Roepstorff, Moller, & Bendixen, 2004). Among the substrates that were degraded by *in vitro* incubation with μ-calpain were desmin, actin, myosin heavy chain, myosin light chain I, troponin T, tropomyosin α1, tropomyosin α4, thioredoxin and CapZ.

Changes in metabolic protein composition in biopsies from live animals to *post-mortem* samples collected shortly after slaughter in the cattle LD muscle revealed that 24 protein spots were changed (Jia et al., 2006). This reflects the contribution of several factors such as transportation, lairage, stunning, exsanguination and dehiding on the LD muscle proteome. Identification of the proteins by MALDI-TOF/TOF MS revealed that a wide range of metabolic enzymes and stress proteins increased in abundance after slaughter. Several of these proteins were glycolytic enzymes such as enolase, aldehyde dehydrogenase, phosphoglycerate kinase or enzymes involved in oxidative metabolism such as ATP-specific succinyl-CoA synthetase and isocitrate dehydrogenase. This supports an expected shift in energy metabolism in the muscle *post-mortem* via the glycolytic pathway, and also an increase in aerobic energy metabolism the first hour after slaughter.

Changes in the muscle proteome between slaughter and 24 h storage in bovine LD and ST muscles was observed both between the sampling times and between the LD and ST muscles (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006). In this study 5 proteins were changed in both muscles, namely cofilin, lactoylglutathione lyase, substrate protein of mitochondrial ATP-dependent proteinase SP-22, HSP27KDa and HSP20KDa. However, 15 proteins were changed in either LD or ST muscles. These differences reflect distinct metabolic and physiological functions of the different muscles.

3.4. Proteome changes due to pre-slaughter conditions

During the last few years, proteomics has also been applied to investigate proteome changes induced by different pre-slaughter conditions. The first example involves a study of compensatory growth in pigs, which has been associated with more tender meat (Kristensen, Therkildsen, Aaslyng, Oksbjerg, & Ertbjerg, 2004). In a proteome study of pigs at slaughter, seven proteins were changed according to compensatory growth. Among them several stress proteins and glycolytic proteins were decreased in abundance (Lametsch et al., 2006). In the same study eight proteins were affected 48 h after slaughter including both structural proteins and enzymes.

In another study, the effect of *post-mortem* storage time (0, 12, and 72 h) and different pre-slaughter treatments (transported immediately before slaughter vs. transported 12 h before slaughter) of pigs on the LD muscle proteome were analysed (Morzel et al., 2004). Most differences were found as a result of *post-mortem* storage, and 37 spots varied with storage time. Ten of these increased in abundance during the storage period, such as fragments of actin, troponin T, and α-crystallin resulting from proteolysis. The pre-slaughter treatments affected 8 protein spots, while they had no effect on the proteolytic events taking place during the storage period.

3.5. Proteome changes associated with meat quality traits

In a study of proteome changes related to tenderness (Warner Bratzler shear force) in pork LD muscle 6 proteins were affected (Lametsch et al., 2003). A great increase of fragments of actin during *post-mortem* storage was observed. Three actin fragments as well as a myosin heavy chain fragment were correlated to shear force. Moreover, myosin light chain II and the glycolytic enzyme triose phosphate isomerase were correlated to tenderness. This has also been confirmed by Hwang (Hwang, Park, Kim, Cho, & Lee, 2005). In another study, proteome analysis of SM muscle from normal hams and from PSE-zones of defective hams demonstrated a reduced proteolysis of troponin T, MLC 1, and α-crystallin in the defect muscles (Laville et al., 2005).

Recently, 2-DE and MS have also been used to investigate the biochemical mechanisms behind variation in meat colour (Hwang et al., 2005; Sayd et al., 2005). Comparison of the sarcoplasmic proteome in pig SM muscle from two groups of animals having a light or dark meat colour revealed that 22 protein spots were differentially expressed (Sayd et al., 2006). The animals, 12 animals in each group, were selected from samples of 1000 pigs based on extreme L^*-values. While the dark muscles had an increased abundance of mitochondrial proteins, indicating a more oxidative metabolism, the light muscles had an increased abundance of cytosolic proteins involved in glycolysis.

Proteome analyses have also been used to study protein changes in dry-cured hams. In one study, the myofibrillar proteins of raw meat and dry-cured hams after 6, 10 and 14 months of ripening were analysed by 2-DE (Di Luccia et al., 2005). Both actin, tropomyosin and myosin light chain disappeared during the ripening period, and were almost completely hydrolysed after 12 months. In a pilot study of Norwegian dry-cured hams we have earlier observed a variation in the protein degradation pattern between hams ripened for 6 months from different producers (Sidhu, Hollung, & Berg, 2005).

3.6. MS-analysis of peptides

The majority of proteomics studies in meat science are based on a combination of 2-DE and MS. However, recently

the occurrence of low-molecular weight peptides in bovine *pectoralis profundus* muscle during post-mortem storage and cooking were analysed directly by MS (Bauchart et al., 2006). Samples were taken approximately 30 min after slaughter (T0) and after 14 days of ageing. On day 14 samples were taken from both cooked (T14c) and uncooked (T14) muscle. These extracts were subsequently analysed for amino acid composition, and a combination of MALDI-TOF MS and nano-LC-ion trap MS/MS analyses were used to determine peptide composition and identification, respectively. The peak patterns from the MALDI-TOF MS analysis were very reproducible between animals, and 7, 18 and 92 peptides were detected at T0, T14, and T14c, respectively. None of the peptides found at T0 and T14 were identified, however seven peptides corresponding to 5 different proteins were identified from the T14c samples. Three of these proteins are known targets of *post-mortem* proteolysis (troponin T, nebulin, cypher protein) (Geesink & Koohmaraie, 1999; Morzel et al., 2004), while the other two proteins were the connective tissue proteins procollagen types I and IV. These are known to be very stable during meat ageing, however prolonged heating above 70 °C can promote collagen peptide bond breakage and explain the occurrence of these peptides in the cooked samples.

4. Future prospects

Proteomics has turned out to be a promising and powerful tool in meat science over the last years yielding information on differences between muscles in a carcass. Furthermore, the combined approach of 2-DE and MS has demonstrated a great strength in studying metabolic alterations, *post-mortem* proteolysis, and changes induced by environmental and processing conditions. As a result an increasing number of studies are emerging in the literature using proteomics as the key tool to unleash the molecular mechanisms behind different genetic backgrounds or processing techniques of meat. Although the genetic background is important, the major contribution to meat quality is caused by processing and environmental conditions. Thus proteomics could be the tool to reflect the important mechanisms and contributions to development of a satisfactory meat quality. In contrast to traditional methods where only one or a few proteins are studied at a time, several hundred proteins can be studied simultaneously by proteome analysis. By manipulations with the protein extraction, different proteins are enriched and this should be carefully considered before designing the experiments. By proteomics it is possible to investigate proteins that originally were not included in the hypothesis of the experiment and may thereby provide answers leading to new hypotheses according to a certain treatment or quality. At present, there has been focus on development of techniques and mapping of proteomes according to genotypes and muscle types. In the future, focus should be directed towards understanding variation and finding markers for meat quality traits.

While proteomics yields important information by itself, the potential in linking information generated by this technique with other –omics techniques is vast. An integrated functional genomics approach can be used to monitor quantitative and qualitative differences in the transcriptome, proteome, and metabolome, creating a powerful tool to study gene function and cellular responses to external stimuli. In meat science, this approach will be of great importance, since meat quality is highly influenced by external stimuli such as environmental and processing conditions. However, linking and extracting information from such large data matrices is a formidable task, and will require significant research attention in the coming years. This implies diligent coordination between scientists that masters the techniques and analyses in the different fields. If we are successful in this endeavour, the future for proteomics in meat research is bright. Proteomics has the potential to shift the understanding of molecular mechanisms underlying meat quality a great leap forward.

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References

Aebersold, R. (2003). A mass spectrometric journey into protein and proteome research. *Journal of the American Society for Mass Spectrometry, 14*, 685–695.

Aebersold, R., & Mann, M. (2003). Mass spectrometry-based proteomics. *Nature, 422*, 198–207.

Bandman, E., & Zdanis, D. (1988). An immunological method to assess protein-degradation in post-mortem muscle. *Meat Science, 22*, 1–19.

Bauchart, C., Remond, D., Chambon, C., Mirand, P. P., Savary-Auzeloux, I., Reynes, C., et al. (2006). Small peptides (<5 kDa) found in ready-to-eat beef meat. *Meat Science, 74*, 658–666.

Bendixen, E. (2005). The use of proteomics in meat science. *Meat Science, 71*, 138–149.

Bendixen, E., Taylor, R., Hollung, K., Hildrum, K. I., Picard, B., & Bouley, J. (2005). Proteomics, an approach towards understanding the biology of meat quality. In J. F. Hocquette & S. Gigli (Eds.), *Indicators of milk and beef quality* (Vol. 112, pp. 81–94). Wageningen: Wageningen Academic Publishers.

Bodenmiller, B., Mueller, L. N., Mueller, M., Domon, B., & Aebersold, R. (2007). Reproducible isolation of distinct, overlapping segments of the phosphoproteome. *Nature Methods, 4*, 231–237.

Bouley, J., Chambon, C., & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics, 4*, 1811–1824.

Bouley, J., Meunier, B., Chambon, C., De Smet, S., Hocquette, J. F., & Picard, B. (2005). Proteomic analysis of bovine skeletal muscle hypertrophy. *Proteomics, 5*, 490–500.

Di Luccia, A., Picariello, G., Cacace, G., Scaloni, A., Faccia, M., Liuzzi, V., et al. (2005). Proteomic analysis of water soluble and myofibrillar protein changes occurring in dry-cured hams. *Meat Science, 69*, 479–491.

Fey, S. J., & Larsen, P. M. (2001). 2D or not 2D. Two-dimensional gel electrophoresis. *Current Opinion in Chemical Biology, 5*, 26–33.

Frohlich, T., Helmstetter, D., Zobawa, M., Crecelius, A. C., Arzberger, T., Kretzschmar, H. A., et al. (2006). Analysis of the HUPO Brain Proteome reference samples using 2-D DIGE and 2-D LC–MS/MS. *Proteomics, 6*, 4950–4966.

Geesink, G. H., & Koohmaraie, M. (1999). Postmortem proteolysis and calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended postmortem storage. *Journal of Animal Science, 77*, 1490–1501.

Goll, D. E., Taylor, R. G., Christiansen, J. A., & Thompson, V. F. (1991). Role of proteinases and protein turnover in muscle growth and meat quality. *Proceedings of the Reciprocal Meat Conference, 44*, 25–33.

Gorg, A., Obermaier, C., Boguth, G., Harder, A., Scheibe, B., Wildgruber, R., et al. (2000). The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis, 21*, 1037–1053.

Gorg, A., Weiss, W., & Dunn, M. J. (2004). Current two-dimensional electrophoresis technology for proteomics. *Proteomics, 4*, 3665–3685.

Grove, H., Hollung, K., Uhlen, A. K., Martens, H., & Faergestad, E. M. (2006). Challenges related to analysis of protein spot volumes from two-dimensional gel electrophoresis as revealed by replicate gels. *Journal of Proteome Research, 5*, 3399–3410.

Hamelin, M., Sayd, T., Chambon, C., Bouix, J., Bibe, B., Milenkovic, D., et al. (2006). Proteomic analysis of ovine muscle hypertrophy. *Journal of Animal Science, 84*, 3266–3276.

Hildrum, K. I., & Tornberg, E. (1998). *Nordfood: Managing the meat tenderization process: A Nordic cooperation project between Norwegian and Swedish meat industry and research institutes*. Oslo: Nordic Industrial Fund.

Hopkins, D. L. & Taylor, R. G. (2002). Post-mortem muscle proteolysis and meat tenderness.

Hufflonergan, E., Parrish, F. C., & Robson, R. M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. *Journal of Animal Science, 73*, 1064–1073.

Hwang, I. H., Park, B. Y., Kim, J. H., Cho, S. H., & Lee, J. M. (2005). Assessment of postmortem proteolysis by gel-based proteome analysis and its relationship to meat quality traits in pig longissimus. *Meat Science, 69*, 79–91.

Jacobsen, S., Grove, H., Jensen, K. N., Sørensen, H. A., Jessen, F., Hollung, K., et al. (2007). Multivariate analysis of two-dimensional gel electrophoresis protein patterns - Practical approaches. *Electrophoresis, 28*, 1289–1299.

Jessen, F., Lametsch, R., Bendixen, E., Kjaersgard, I. V., & Jorgensen, B. M. (2002). Extracting information from two-dimensional electrophoresis gels by partial least squares regression. *Proteomics, 2*, 32–35.

Jia, X., Hildrum, K. I., Westad, F., Kummen, E., Aass, L., & Hollung, K. (2006). Changes in enzymes associated with energy metabolism during the early post-mortem period in longissimus thoracis bovine muscle analyzed by proteomics. *Journal of Proteome Research, 5*, 1763–1769.

Jia, X., Hollung, K., Therkildsen, M., Hildrum, K. I., & Bendixen, E. (2006). Proteome analysis of early post-mortem changes in two bovine muscle types: *M. longissimus dorsi* and *M. semitendinosis*. *Proteomics, 6*, 936–944.

Kjaersgard, I. V., Norrelykke, M. R., & Jessen, F. (2006). Changes in cod muscle proteins during frozen storage revealed by proteome analysis and multivariate data analysis. *Proteomics, 6*, 1606–1618.

Koohmaraie, M. (1994). Muscle proteinases and meat aging. *Meat Science, 36*, 93–104.

Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Science, 43*, S193–S201.

Kristensen, L., Therkildsen, M., Aaslyng, M. D., Oksbjerg, N., & Ertbjerg, P. (2004). Compensatory growth improves meat tenderness in gilts but not in barrows. *Journal of Animal Science, 82*, 3617–3624.

Lametsch, R., & Bendixen, E. (2001). Proteome analysis applied to meat science: Characterizing postmortem changes in porcine muscle. *Journal of Agricultural and Food Chemistry, 49*, 4531–4537.

Lametsch, R., Karlsson, A., Rosenvold, K., Andersen, H. J., Roepstorff, P., & Bendixen, E. (2003). Postmortem proteome changes of porcine muscle related to tenderness. *Journal of Agricultural and Food Chemistry, 51*, 6992–6997.

Lametsch, R., Kristensen, L., Larsen, M. R., Therkildsen, M., Oksbjerg, N., & Ertbjerg, P. (2006). Changes in the muscle proteome after compensatory growth in pigs. *Journal of Animal Science, 84*, 918–924.

Lametsch, R., Roepstorff, P., & Bendixen, E. (2002). Identification of protein degradation during post-mortem storage of pig meat. *Journal of Agricultural and Food Chemistry, 50*, 5508–5512.

Lametsch, R., Roepstorff, P., Moller, H. S., & Bendixen, E. (2004). Identification of myofibrillar substrates for mu-calpain. *Meat Science, 68*, 515–521.

Laville, E., Sayd, T., Sante-Lhoutellier, V., Morzel, M., Labas, R., Franck, M., et al. (2005). Characterisation of PSE zones in semimembranosus pig muscle. *Meat Science, 70*, 167–172.

Luhn, S., Berth, M., Hecker, M., & Bernhardt, J. (2003). Using standard positions and image fusion to create proteome maps from collections of two-dimensional gel electrophoresis images. *Proteomics, 3*, 1117–1127.

Martens, H., & Martens, M. (2000). Modified Jack.knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). *Food Quality and Preference, 11*, 5–16.

Maurer, M. H., Feldmann, R. E., Jr., Bromme, J. O., & Kalenka, A. (2005). Comparison of statistical approaches for the analysis of proteome expression data of differentiating neural stem cells. *Journal of Proteome Research, 4*, 96–100.

McDonald, T., Sheng, S., Stanley, B., Chen, D., Ko, Y., Cole, R. N., et al. (2006). Expanding the subproteome of the inner mitochondria using protein separation technologies: one- and two-dimensional liquid chromatography and two-dimensional gel electrophoresis. *Molecular and Cellular Proteomics, 5*, 2392–2411.

Melody, J. L., Lonergan, S. M., Rowe, L. J., Huiatt, T. W., Mayes, M. S., & Huff-Lonergan, E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. *Journal of Animal Science, 82*, 1195–1205.

Meunier, B., Dumas, E., Piec, I., Bechet, D., Hebraud, M., & Hocquette, J. F. (2007). Assessment of hierarchical clustering methodologies for proteomic data mining. *Journal of Proteome Research, 6*, 358–366.

Miller, M. F., Huffman, K. L., Gilbert, S. Y., Hamman, L. L., & Ramsey, C. B. (1995). Retail consumer acceptance of beef tenderized with calcium chloride. *Journal of Animal Science, 73*, 2308–2314.

Morzel, M., Chambon, C., Hamelin, M., Sante-Lhoutellier, V., Sayd, T., & Monin, G. (2004). Proteome changes during pork meat ageing following use of two different pre-slaughter handling procedures. *Meat Science, 67*, 689–696.

Næs, T., Isaksson, T., Fearn, T., & Davies, T. (2002). *A user-friendly guide to multivariate calibration and classification*. Chichester: NIR Publications.

Rabilloud, T. (2002). Two-dimensional gel electrophoresis in proteomics: old, old fashioned, but it still climbs up the mountains. *Proteomics, 2*, 3–10.

Reinders, J., Lewandrowski, U., Moebius, J., Wagner, Y., & Sickmann, A. (2004). Challenges in mass spectrometry-based proteomics. *Proteomics, 4*, 3686–3703.

Righetti, P. G., Castagna, A., Antonioli, P., & Boschetti, E. (2005). Prefractionation techniques in proteome analysis: the mining tools of the third millennium. *Electrophoresis, 26*, 297–319.

Sayd, T., Morzel, M., Chambon, C., Franck, M., Figwer, P., Larzul, C., et al. (2006). Proteome analysis of the sarcoplasmic fraction of pig semimembranosus muscle: Implications on meat color development. *Journal of Agricultural and Food Chemistry, 54*, 2732–2737.

Sidhu, M. S., Hollung, K., & Berg, P. (2005). Proteolysis in Norwegian dry-cured hams; preliminary results. In *51th ICOMST – the international congress of meat science and technology, Baltimore, USA*.

Stasyk, T., & Huber, L. A. (2004). Zooming in: Fractionation strategies in proteomics. *Proteomics, 4*, 3704–3716.

Steen, H., & Mann, M. (2004). The ABC's (and XYZ's) of peptide sequencing. *Nature Reviews Molecular Cell Biology, 5*, 699–711.

Taylor, R. G., Geesink, G. H., Thompson, V. F., Koohmaraie, M., & Goll, D. E. (1995). Is Z-disk degradation responsible for postmortem tenderization. *Journal of Animal Science, 73*, 1351–1367.

Wilkins, M. R., Pasquali, C., Appel, R. D., Ou, K., Golaz, O., Sanchez, J. C., et al. (1996). From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. *Biotechnology (NY), 14*, 61–65.