Current advances in proteomic analysis and its use for the resolution of poultry meat quality problems

H. RÉMIGNON*, C. MOLETTE and R. BABILE

Department of Animal Science, Ecole Nationale Supérieure Agronomique de Toulouse, BP107, 31326 Castanet-Tolosan, France
Laboratory supported by INRA-PHASE
***remignon@ensat.fr**

Keywords: proteome analysis; poultry meat quality; electrophoresis

Abstract

If proteins are the functional units of almost all biological processes, the proteome represents the set of proteins expressed in a cell at a given time and for given conditions. Because muscle is mainly composed of water and proteins, it seems evident that proteome analysis can give much information on the structure and functions of proteins involved in several mechanisms which determine the quality of the meat.

This paper aims to present the most classical techniques (i.e. sample preparation and protein solubilization, protein separation by 2-DE or SDS-Page, protein detection and quantitation, computer analysis of 2DE-pattern and finally protein identification) that are currently applied to analyse the proteome.

Proteomic study related to meat quality are somewhat limited, especially in poultry, but some results are presented to illustrate how proteomic approaches can bring a new point of view on new or already known meat quality problems. It is the case in mammals where tenderness of the meat had been given a new highlight with proteomic study but also in turkeys where new hypothesis for explaining PSE meat syndrome can be developed from proteomic analysis. Some studies have also reported interested results in muscle growth and development in chickens.

The quality of the meat is a complex problem which includes several factors of variation such as genetic, handling of animals during production, transportation and slaughter and also the handling of the meat during product processing. Proteomic tools can give a new point of view on these problems and help the biologist to understand and finally resolve it.

Introduction

During the last decade, genomic studies transformed biological research and of course meat quality studies, but relationships between genes and their proteins or “proteome” of a cell are not strictly linear. So, proteomic is complementary to genomic because it focuses on gene products (as functional genomic) being active agents in cells. For example, in muscles, proteins sustain function, not genes (Kim *et al.*, 2004). In the last years, proteomic studies tended to be more and more popular, beside genomic studies, and are now often cited as a wonderful promising tool which could help many biologists to resolve numerous problems in their field of interest: one of these could be poultry meat quality.

It is commonly admitted that proteomics is a large-scale study of proteins, usually by biochemical methods (Pandley and Mann, 2000). More practically, proteomic is generally achieved with the study of large quantities of proteins on two dimensional polyacrylamide gels.

Proteomic in practice

Bi-dimensional electrophoresis (2-DE) combined with protein identification by mass spectrometry (MS) is generally used for proteomic analyses. 2-DE-MS major steps include the following (for a review see, Görg *et al.*, 2004).

SAMPLE PREPARATION

The tissue has first to be disaggregated to have a full access to the different cell components. To ensure a good quality of 2-DE-MS, proteases (very important in muscle tissue) have to be inactivated, interfering substances (nucleic acids, lipids, polysaccharides…) have to be removed and proteins solubilized. All these steps are necessary to prepare individual proteins without molecular interactions. Several ways are possible to achieve these goals and are well documented in the literature.

TWO DIMENSIONAL ELECTROPHORESIS

Since the first papers on 2-DE by O'Farrell (1975), recent technological advances made 2-DE more efficient and reproducible. The basic principles remain to first separate the proteins according to their isoelectric point (pI) in a step called isoelectrofocalisation (IEF). This is actually done with Immobilized pH Gradients (IPG) strips which allowed very accurate separations of proteins with a high reproducibility. In a second step, proteins are separated according to their molecular weight in a classical SDS-PAGE electrophoresis.

PROTEIN DETECTION AND QUANTITATION

Different staining procedures are available to detect proteins in electrophoresis gels. These methods are more or less sensitive (to allow detection of very minor proteins) but must always be compatible with post-electrophoretic protein identification procedures such as mass spectrometry. Consequently, gels are digitalised and computer image analysis procedures are used to identify differences or similarity between different sets of protein samples. Ünlü *et al.* (1997) proposed a new method (called DIGE) in which proteins extracted from two different samples are labelled in vitro with two different fluorescent cyanine dyes. Finally, proteins are separated and visualized on one same gel which facilitates the identification of differences (quantitative and/or qualitative) in proteins patterns between the two samples.

PROTEIN IDENTIFICATION FROM 2-DE GEL SPOTS

After computer image analysis, spots (or proteins) of interest can be excised from 2DE-gels and further processed (including trypsin digestion) before their final identification. MS became the technique of reference for such identification. Mass spectrometer as MALDI-TOF (Matrix-Assisted Laser Desorption Ionization Time-of-Flight) or ESI-MS (Electrospray Ionization / Mass Spectrometry) technologies are now currently used to get protein mass fingerprint (PMF) which allow protein identification from informations stored in databases. Recent advances in MS also allow the investigation of post-traductional modifications (PTM) such as phophorylation and glycosylation. This recent advance makes a big difference between proteomic and genomic approaches since the latter does not provide informations about PTM which can be essential for protein functionalities.

More and more proteomic cards from different animals and/or tissues are available but they only produce references for collecting informations on presence or absence of some particular proteins in given tissues (protein mapping) .To try to compare two or more sets of samples collected from different situations (low *vs.* high quality meat for example), the most popular way used is the comparison of two samples of proteins migrated in the same conditions but on different gels (Figure 1), or on the same gel but with specific labels on the proteins extracted from different samples (DIGE System, 2-D Fluorescence Difference Gel Electrophoresis)

Proteomic and meat quality

As more than 20 % of muscle (or meat) mass is composed with proteins, it seems somewhat evident that a link between muscle proteins and meat quality can be drawn. Nevertheless, nowadays, few proteomic studies related to a precise meat quality defect are available. On the contrary, it is easier to find papers relating changes in proteome pattern related to muscle growth or transformation during post-mortem modifications. In mammals, proteomic studies particularly focused on tenderness properties of meat because it is a permanent problem in beef and, to a less extend, in pork.

Lametsch and Bendixen (2001) first demonstrated the interest of proteome analysis to characterize post mortem changes in porcine muscles. They report 15 notable changes in proteome patterns (muscle proteins of 5-200 kDa, pH span of 4-9) between samples taken from slaughter to 48 h post

mortem. In the following papers, (Lametsch *et al.*, 2002 and 2003), they finally identified more than 20 proteins which can be used as meat quality markers. Among them, fragments of actin and myosin heavy chain (MHC) were found to be significantly correlated to shear force value which clearly indicates that the post-mortem degradation of actin and MHC influences meat texture. In a meat quality study concerning the carriers of the porcine RN^- mutation, Hedegaard *et al.* (2004) demonstrated that proteomic analysis of muscle proteins gave new informations on metabolic disorders associated with this syndrome.

Bouley *et al.* (2005) used proteomic to study bovine skeletal muscle hypertrophy and reported that the related myostatin deletion induced alteration in thirteen muscular proteins. This study is of particularly interest because it reported protein alterations which were not directly related to myostatin. In consequence, it could not have been identified as the responsible factor for double muscling syndrome with proteomic study while it was with the genomic approach.

Proteomic and poultry meat quality

Unfortunately, there are still very few studies reporting results concerning poultry muscle development and/or meat quality. Concerning muscle development a paper published by Doherty *et al.* (2004) reported that characterisation of the proteome of layer chicken pectoralis muscle showed dramatic changes in relative expression levels of several proteins all along the growth. This work indicated the complexity in such analysis because isoenzyme shifts, associations with structural elements and post-translational modifications all characterize growth in the muscle system. Nevertheless, it was a first interesting step towards a better understanding of muscle modifications, in poultry, during growth.

Our lab initiated in 2002 proteomic studies in order to better understand the origin of PSE meat syndrome in turkeys. These studies were directed towards muscle protein fractions analysis because if most of the authors, reporting on PSE syndrome in poultry, agreed on a probable origin of the defects due to proteins alterations, very few knowledge on how and which proteins are modified is available. Molette *et al.* (2003 and 2005) reported that turkeys (BUT9) exhibiting fast post-mortem glycolysis in breast muscle (-0.5 pH unit at 20 min post mortem) presented meat quality alterations such as lower water holding capacity, lower processing yield, lower tenderness. They also reported that meat from (FG) fast glycolysing animals had a lower buffering capacity and a lower sarcoplasmic protein extractability suggesting modifications of protein functionality when the rate of pH decline was accelerated. The use of simple SDS-PAGE electrophoresis of different protein fractions was unable to detect differences in the different banding patterns. Subsequently, 2DE (whole muscle protein extracts, IEF between pH 5 and 8) were performed. The differences between the two 2DE gels were retained only on a basis of presence or absence of spots in FG or NG (normal glycolysing) samples. This allowed identification of three spots (figure 2) which were present in NG muscles and missing in FG muscles. These proteins were identified, by using Q-Trap mass spectrometer after trypsin digestion, as myosin heavy chain and actin fragments and glyceraldehyde-3 phosphate dehydrogenase (GAPDH) enzyme. All the samples were collected at a time (24 h post mortem) when the meat aging is not completely achieved. The presence of myofibrillar proteins fragments only in NG muscles indicated that the meat aging seemed to be active earlier or faster in NG than in FG muscles. This result was in good agreement with results obtained for meat tenderness in the present experiment and in PSE pork meats where meat aging is supposed to be lowered (Monin *et al.*, 1999).

In muscle, the metabolic enzymes are mainly sarcoplasmic proteins which exhibit a basic pI. Due to their low solubility, basic proteins are hardly well-separated with 2DE gels. To avoid this drawback, we first decided to separate basic proteins from acid and neutral ones by using ion exchange columns with increasing salt concentrations. Consequently, basic proteins were separated according to their molecular weight in SDS-PAGE conditions and stained with Coomassie brilliant blue (CBB R-250). There was no difference in the banding pattern between NG and FG animals but we can notice major differences in band intensity between the two groups. The two proteins were identified using MALDI-TOF-MS as being fructose biphosphate aldolase (aldolase A) and GAPDH. These two proteins are involved in the first steps of glycolysis pathway. The differences in the staining intensity of the two bands can be interpreted in two ways. First, we can suppose that these two proteins are present in higher quantities in FG than in NG. Secondly, a modification in protein structure can be imagined (modification of the amino acid sequence, change in a prosthetic group …) leading do different reactions of the modified proteins with CBB. For the moment, neither of these two hypothesis can be retained (further investigations are needed) but they all abound in the way of a probable modification in the glycolysis pathway in FG muscles.

Conclusions

Our first results obtained with PSE-like turkey meats examined with proteomic tools finally indicated that meat aging was probably modified in FG muscles and this could be linked to modifications in proteolytic enzymes functionalities and/or to muscle proteins denaturations which would prevent proteases to act normally. On the other hand, the possible modifications of two glycolytic enzymes could explain the observed differences in the rate of pH decline. Complementary, proteomic analyses could be used to try to detect, as earlier as possible, animals, carrying these modifications; which are supposed to be prone to give this lower meat quality.

From a more general point of view, we can hope, in a short future, new informations on poultry meat quality defect origins will be accessible favour to proteomic tools. This is largely due to the recent technical developments in 2DE and to facilitated access to MS technology. Nevertheless, we must remember that proteomic is a tool not a solution. In consequence, all meat quality studies must not necessary include proteomics while those including them will not always lead to the most relevant results.

References

BOULEY J., MEUNIER B., CHAMBON C., DE SMET S., HOCQUETTE J-F. AND PICARD B. (2005). Proteomic analysis of bovine skeletal muscle hypertrophy. Proteomics,5 (2), 490-500.

DOHERTY M.K., MCLEAN L., HAYTER J.R., PRATT J.M., ROBERTSON D.H.L., EL-SHAFEI A., GASKELL S.J. AND BEYNON R. (2004). The proteome of chicken muscle: Changes in soluble proteins expression during a growth in a layer strain. Proteomics, 4, 2082-2093.

GÖRG, A., WEISS W. AND DUNN, M.J. (2004). Current two-dimensional electrophoresis technology for proteomics. Proteomics, 4, 3665-3685.

HEDEGAARD J., HORN P., LAMETSCH R., SONDERGAARD MOLLER H., ROEPSTORFF P., BENDIXEN C. AND BENDIXEN E. (2004). UDP-Glucose pyrophosphorylase is upregulated in carriers of the porcine RN^- mutation in the AMP-activated protein kinase. Porteomics, 4, 2448-2454.

KIM, N-K., JOH J-H., PARK, H-R., KIM, O-H., PARK, B-E. AND LEE, C-S. (2004). Differential expression profiling of the proteomes and their mRNA in porcine white and red muscles. Proteomics, 4, 3422-3428.

LAMETSCH, R. AND BENDIXEN E. (2001). Proteome analysis applied to meat science: Characterizing post mortem changes in porcine muscle. Journal of Agricultural and Food Chemistry, 49, 4531-4537.

LAMETSCH R., ROEPSTORFF P. AND BENDIXEN E. (2002). Identification of protein degradation during post-mortem storage of pig meat. Journal of Agricultural and Food Chemistry, 52, 5508-5512. Journal of Agricultural and Food Chemistry, 51, 6992-6997.

LAMTESCH R., KARLSSON A., ROSENVOLD K., ANDERSEN H.J., ROEPSTORFF P. AND BENDIXEN E. (2003). Postmortem changes of porcine muscle related to tenderness.

O'FARRELL, P.H. (1975). High resolution two-dimensional electrophoresis of proteins Journal of Biological Chemistry, 250, 4007-4021.

MONIN, G., LARZUL, C., LE ROY, P., CULIOLI, J., MOUROT, J., ROUSSET-AKRIM, S., TALMANT, A., TOURAILLE, C., SELLIER, P. (1999). Effects of the halothane genotype and slaughter weight on texture of pork. Journal of Animal Science, 77, 408-415.

MOLETTE C., RÉMIGNON H. AND BABILÉ R. (2003). Early post-mortem pH and turkey breast meat quality. Proceeding of the XVI European Symposium on the Quality of Poultry Meat, Saint-Brieuc (France), September 23-23, 48-53.

MOLETTE C., RÉMIGNON H. AND BABILÉ R. (2005). Modifications of glycolyzing enzymes induces a lowest meat quality in turkey. Poultry Science, 84, 119-127.

PANDLEY, A. AND MANN, A. (2000). Proteomics to study genes and genomes. Nature, 405, 837-846.

ÜNLÜ, M., MORGAN, M.E. AND MINDEN J.S. (1997). Difference gel electrophoresis: a single gel method for detecting changes in cell extracts. Electrophoresis, 18, 2071-2077.

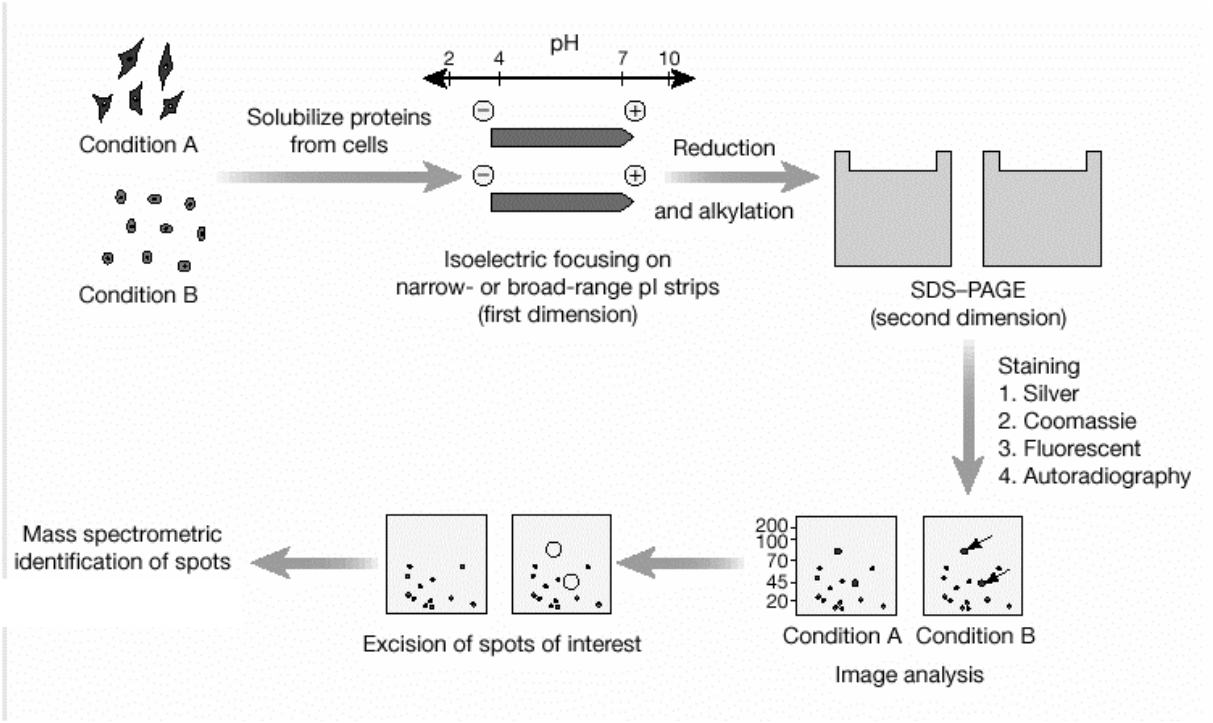

Figure 1 2-DE principles applied to the comparison of two types of samples (Source: http://cmbi.bjmu.edu.cn).

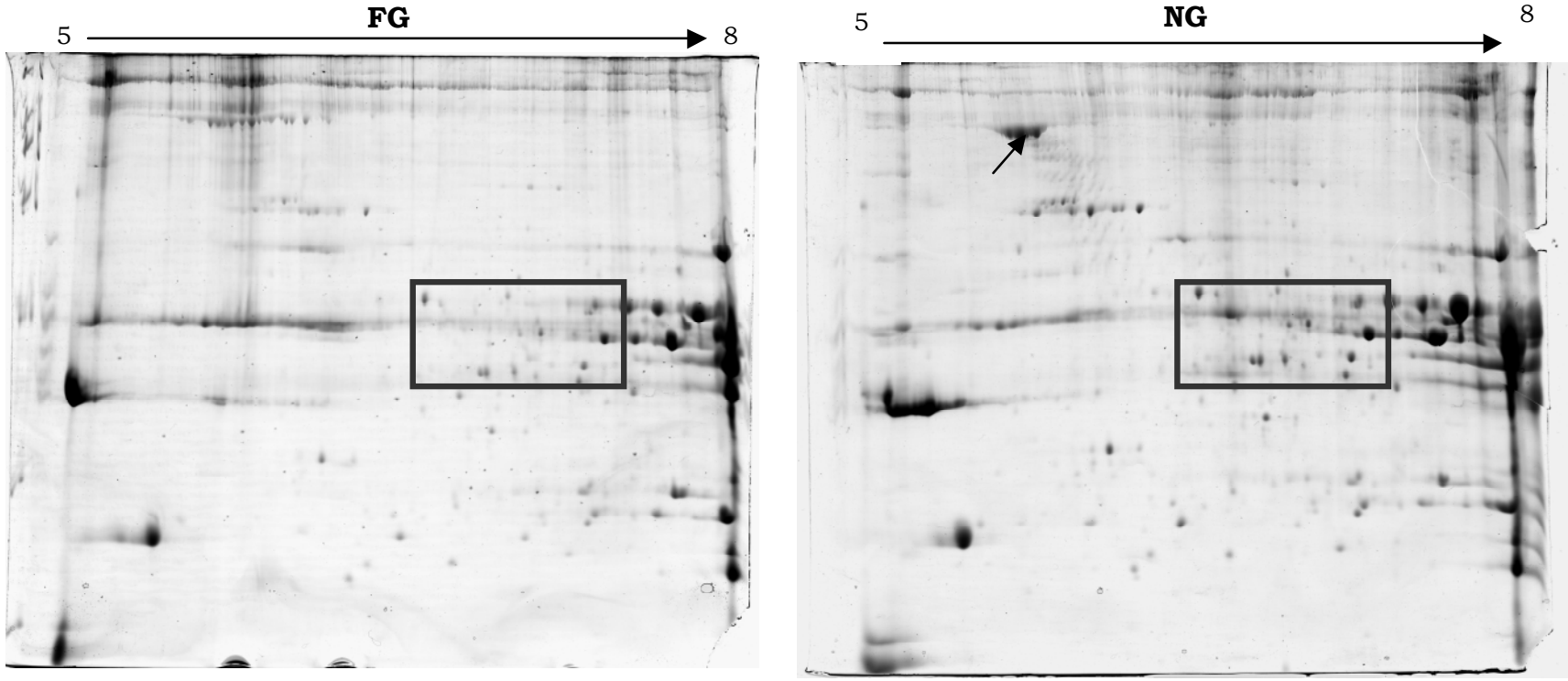

Figure 2 2-DE gels obtained from beast muscle extracts. The arrows indicate spots that are only present on gels from NG animals. The rectangle indicates the enlarging zone presented below.