Comparison of muscle proteome profile in Norwegian Landrace, Duroc and Hampshire at three different ages

K. Hollung[1], H. Grove[1, 2], E.M. Færgestad[1], M.S. Sidhu[3] & P. Berg[3]
[1]Nofima Food, Matforsk AS - Food, Fisheries and Aquaculture Research, Osloveien 1, N-1430 Ås, Norway.
[2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway.
[3]Animalia - Norwegian Meat and Poultry Research Centre, P.O. Box 396 Økern, N-0513 Oslo, Norway.
E-mail: kristin.hollung@matforsk.no.

Abstract

We have used proteomics as a tool to unravel the changes in protein composition between three pure pig breeds and age groups in this study. Fifty female pigs of the Norwegian Landrace, Duroc and Hampshire breed slaughtered at 6, 9 and 12 months age were included in the study. Each of three breeds was raised in three separate farms and was slaughtered at the same day in a commercial abattoir. A sample from the adductor muscle was collected approximately 45 min post-mortem. Proteome analyses of the soluble proteins using 2-D electrophoresis showed that of the 1125 analyzed protein spots, 51 proteins and 91 proteins are changed in abundance according to age and breed, respectively. A total number of 73 changed proteins were identified by mass spectrometry. The identified proteins can be classified as structural proteins, metabolic proteins, stress/defense proteins and other proteins. This demonstrates a difference in metabolism and muscle composition between breeds and age groups and shows that proteomics is a useful tool to uncover the molecular basis for physiological differences in muscles between pig breeds and age groups.

Introduction

Meat quality of pork is influenced by a number of factors such as genetics, age and processing conditions. Proteomics is a powerful tool that has been used in several studies to analyze changes in protein composition in muscle related to meat science (Bendixen, 2005; Hollung *et al.*, 2007). The changes in protein profile during tenderization of pig muscle (Lametsch and Bendixen, 2001; Lametsch *et al.*, 2003; Hwang *et al.*, 2005) as well as the influence of pre-slaughter conditions (Morzel *et al.*, 2004) have been characterized in several studies using proteomics tools.

The aim of the present study was to use proteomics to unravel the differences in water soluble proteins in muscle from pigs of different age and breed. To analyze the contribution of each breed separately, we have selected pigs from pure breeds.

Materials & methods

Fifty female pigs of Duroc, Hampshire or Norwegian Landrace breeds at 6, 9 and 12 months age were included in this study. All animals were slaughtered on the same day. Approximately 45 min after exsanguination a sample was excised from the *adductor* muscle, snap frozen in N_2(l) and stored at -80ºC until extraction of sarcoplasmic proteins in a TES buffer (10 mM Tris (pH 7.6), 1 mM EDTA and 0.25 M sucrose). Proteins were labelled with Cy2 or Cy5 and separated by 2-dimensional electrophoresis (2-DE) using IPG 5-8 and 12.5% SDS PAGE. Significance testing was performed by ANOVA using p-values adjusted according to False Discovery Rate (FDR). We have also performed a significance test in a multivariate regression approach (Partial Least Squares (PLS) regression) using the double cross-validation method (Cross Model Validation). Proteins of interest were extracted from the 2-DE gels and identified by Maldi-TOF/TOF mass spectrometry.

Results and discussion

Proteins from the *adductor* muscle from Duroc and Norwegian Landrace were separated by 2-DE using the DIGE technology for protein labeling. A total of 1125 spots were included in the statistical analysis. According to differences between the breeds a total of 94 protein spots were significantly changed. The changed proteins are marked on a representative 2-DE image in Figure 1. Of these, 50 proteins were identified.

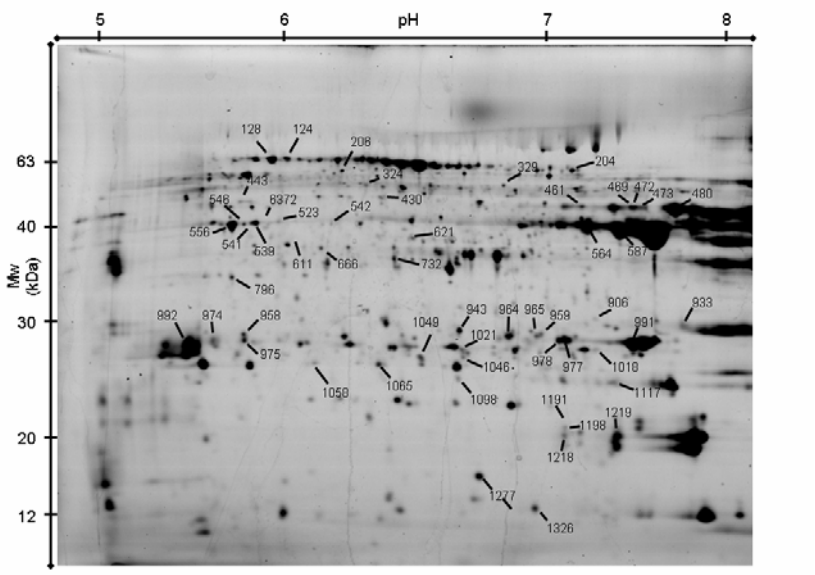

Figure 1. Representative 2-DE DIGE image (Cy 2) of an *adductor* muscle sample. Proteins are separated by pH 5-8 in the first dimension and 12 % SDS-PAGE in the second dimension. Proteins that are significantly changed in abundance between the breeds are marked with numbers.

A total number of 41 protein spots were found to be significantly affected by age (Figure 2). Of these, 21 proteins were identified.

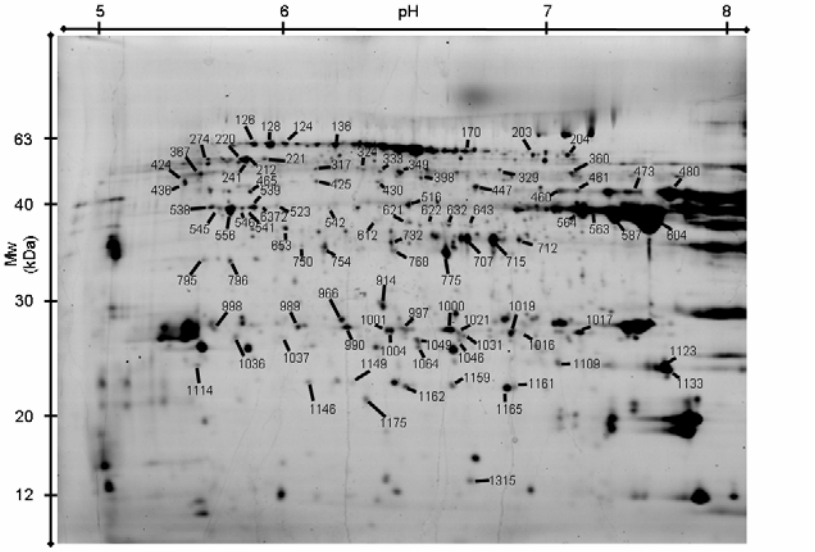

Figure 2. Representative 2-DE DIGE image (Cy 2) of an *adductor* muscle sample. Proteins are separated by pH 5-8 in the first dimension and 12 % SDS-PAGE in the second dimension. Proteins that are significantly changed in abundance according to age are marked with numbers.

Changes in abundance were observed for proteins of different functions such as structural proteins, metabolic proteins, cellular defense and stress proteins as well as several other proteins. In a previous study of these animals we have shown a difference between the breeds at different age groups in several carcass traits and muscle protease activity (Sidhu *et al.*, 2007). Among the structural proteins most of the changed proteins were identified as actin. Norwegian Landrace had lower levels of the identified actin proteins than Duroc and Hampshire. The level of these actin proteins was also lower among the 12 months old pigs than among the younger pigs. A sensory analysis of *longissimus dorsi* of these breeds demonstrated a significant less tender meat in the Norwegian Landrace compared to Duroc and Hampshire (unpublished data). There was also a tendency towards less tender meat among the older pigs compared to the younger pigs. Lower levels of the actin proteins in the 2-DE gels from Norwegian Landrace may reflect that the myofibers are less degraded in these animals thus making the muscle less tender. This is also supported by the findings that

several protein spots of actin are correlated to tenderness in *longissimus dorsi* in a Danish study (Lametsch *et al.*, 2003).

Duroc had higher levels of both HSP27 and crystalline isoforms. These small HSPs are shown to be associated with actin and this fits well with the higher extractability of actin observed among Duroc. The same tendency of increased levels of myofibrillar proteins and HSP27 was found in a pig crossbreed study (Kwasiborski *et al.*, 2008).

Protein spots identified as enolase-3 and G3PDH, involved in the glycolytic pathway were all higher in abundance in the Norwegian Landrace breed. This may reflect a higher glycolytic activity. In addition, the levels of CK were higher in the Norwegian Landrace breed compared to Duroc. CK regulates the intracellular ATP level by reversible phosphorylation of creatine. Taken together this may indicate a higher metabolic rate in the Norwegian Landrace than in the Duroc pigs.

According to age, the levels of 5 TPI protein spots, also involved in glycolysis, are lower in the 6 months old pigs compared to the older pigs. The same pattern is also observed for enolase-3 and malate dehydrogenase. This may reflect a higher glycolytic activity among the older pigs.

Conclusions

We have shown that there are major differences in the abundance of proteins involved in energy metabolism, stress response and structural proteins both between the three breeds studied and between the age groups. This reflects the physiological and meat quality differences observed among these breeds.

Acknowledgement.
This work was supported by a grant from the Norwegian Research Council (No 167942/110).

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