PE1.72 Understanding pork meat quality using proteomic tools 444.00

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***Abstract* — Reducing variability in the eating quality of pork is a major challenge facing the swine industry and requires knowledge of factors ranging from genetics through to post mortem handling. This study investigated the existing variability for a specific meat quality trait, water holding capacity, within a population of 31 pigs. The most extreme animals for this trait were selected for a detailed proteomic study which aims to identify molecular profiles associated with the trait. The proteomic approach compares the protein content of the muscle exudate using 2D electrophoresis followed by mass spectrometric analysis. Using this approach, clear proteomic differences have been identified between exudates from animals divergent for the water holding capacity trait and between exudates collected at different times post mortem. Identification of the specific proteins of interest by mass spectrometry is ongoing and it is hoped that this will aid in the identification of biomarkers that may be of high value to the Irish pork meat industry to deliver high and consistent quality meat to the consumer market.**

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I. Introduction

Meat quality is a complex series of traits that are influenced by individual genetic variation and also by non-genetic factors. Meat scientists have performed substantial research on non-genetic factors such as farm, transport, slaughter and processing conditions and this has led to considerable quality and compositional improvements. There has, to date, been little emphasis on factoring in the molecular or biological components of meat quality into management systems and selection strategies. Many important traits are at least moderately heritable and several genes which influence pork quality have been identified, but considerable variation remains unexplained. One approach to the identification of proteins underlying variation in Irish pork meat quality and to the application of this knowledge in marker-assisted selection strategies is to compare protein expression profiles in animals divergent for meat quality. Previous research in our group has suggested that centrifugal drip would be a suitable substrate to identify biomarkers that may be used to predict and control pork quality in the future (1). Here we present a 2-dimensional proteomic analysis of centrifugal drip samples from animals divergent for the meat quality trait of water holding capacity.

II. MATERIALS AND METHODS

Animal sampling

Thirty one Large White x Landrace/Large White gilts were slaughtered under controlled conditions in a pilot abattoir at the Ashtown Food Research Centre, Dublin. Tissue samples were collected from the longissimus thoracis et lumborum muscle at 1 day, 3 days and 7 days post mortem. Exudates were collected from the muscle tissue following centrifugation (centrifugal drip), where 8g core samples were centrifuged at 7,000 rpm (4ºC) for 60 minutes.

Meat Quality Measurements

A variety of technological quality measurements were performed on the carcasses over a 7 day post slaughter period. Drip loss was measured using a bag method to calculate the percentage drip loss during a 48 hour period (2). pH and temperature were measured at 45 mins, 2, 3, 4, 5, 6 and 24 hours post slaughter. Colour of the semimembranosus muscle was measured (Mini-Scan XE) at 3, 6 and

24 hours post slaughter. Colour of the longissimus thoracis et lumborum muscle was measured at 1, 3 and 7 days post slaughter.

A. Two-Dimensional Gel Electrophoresis (2-DE).

Centrifugal drip samples from a total of 36 biological samples were compared in one experiment using 2-D Fluorescence Difference Gel Electrophoresis (Ettan DIGE) (3). Briefly, each sample was normalised and labelled using Cy5 dye fluor according to GE Healthcare Bio-Sciences AB, Uppsala, Sweden. A pool, to be used as an internal standard, was generated from all 36 samples and this pool was bulk labelled with the Cy3 dye fluor. For each gel, 50 µg of labelled protein from an individual sample plus 50 µg of labelled protein from the pool were mixed together and subjected to 2DE using immobilised pH 4-7 gradients (24cm) in the first dimension and 12% SDS-PAGE in the second dimension (4).

All gels were subsequently scanned using Typhoon (GE Healthcare) (Figure 1a, b). The scanned gels were imported to the software Progenesis SameSpots v2.0 (Nonlinear Dynamics) and then analysed for the detection of spot pattern changes between the different phenotypes and at different times *post mortem* comparisons.

III. RESULTS AND DISCUSSION

A. Identification of individuals divergent in meat quality traits.

Water holding capacity amongst the 31 sampled animals varied approximately 10 fold with the lowest recorded values at 0.9% and the highest at 9.5%. To examine specifically the water holding capacity trait, animals displaying signs of PSE or DFD meat were not selected for detailed proteomic analysis. The drip loss data together with pH45min and Hunter L* measurements were used to detect signs of PSE. Drip loss, pH ultimate and L* colour measurements were used to detect signs of DFD. Of the remaining animals, which did not show signs of PSE or DFD meat, 4 with the highest drip loss, 4 with the lowest drip loss and 4 with intermediate drip loss were selected for further study (Table 1).

Animal	% drip loss	category	Mean % drip loss
1	5.53	high	
2	4.93	high	6.1
3	5.73	high	
4	8.20	high	
5	3.56	intermediate	
6	4.36	intermediate	3.9
7	4.10	intermediate	
8	3.64	intermediate	
9	2.30	low	
10	2.69	low	2.54
11	2.29	low	
12	2.90	low	

Table 1: Selection of 12 animals displaying the most extreme values for drip loss amongst the 31 animals that were sampled.

B. Two-Dimensional Gel Electrophoresis (2-DE).

2-D Fluorescence Difference Gel Electrophoresis (Figure 1a, b) was used to identify specific protein spots differentially expressed between centrifugal drip samples at different times post mortem and from animals displaying divergent meat quality phenotypes. A total of 36 samples were analysed (3 post mortem time points taken from each of the 12 divergent animals listed in Table 1). Significant spot pattern changes were observed between the different comparisons that were made ranging from 10 to 140 spots (see Table 2). Multivariate data analysis using Principal Component Analysis (PCA) Progenesis SameSpots v2.0 (Nonlinear Dynamics) of this data showed distinct grouping which segregated the samples of different phenotypes and from different days post mortem (Figure 2). Ongoing work is focussed on mass spectrometric analysis to determine the identity of the protein spots of interest.

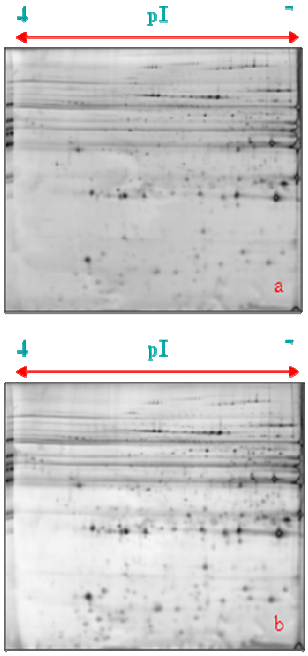

Figure 1 a, b: 2 DE analysis of porcine centrifugal drip sample from the pool sample (a) (all samples from different phenotypes and days *post mortem*) and from a centrifugal drip from an animal with intermediate drip loss at day 3 (b).

50 µg of proteins of the pool sample were labelled with Cy 3 fluor and then mixed with 50 µg of proteins labelled with Cy 5 fluor from the individual sample, then subjected together to 2 DE using immobilised pH 4-7 gradients (24 cm, linear) in the first dimension and 12 % SDS-Page in the second dimension.

	days 1 – 3 - 7
high	79
inter	136
low	66

	high – inter - low
1day	20
3day	10
7day	18

Table 2: Number of protein spots significantly differentially regulated between different groups. high: high drip loss, inter: intermediate drip loss, low: low drip loss.

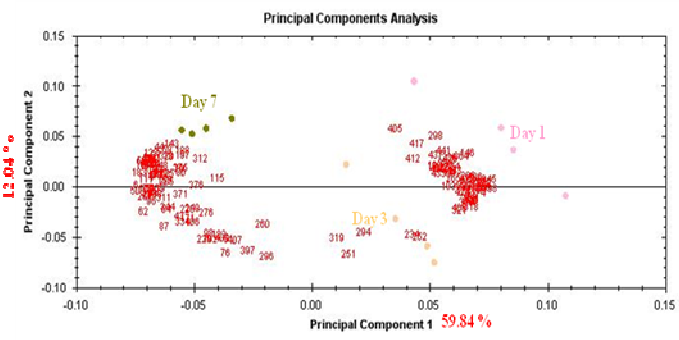

Figure 2: Illustration of the Principal Component Analyses (PCA) carried out using proteins from an animal with an intermediate phenotype for drip loss influenced by time post mortem (days 1, 3 & 7). Distinct grouping are evident at different times post mortem.

IV. Conclusion

The detection of protein changes using 2-D Fluorescence Difference Gel Electrophoresis between centrifugal drip samples verifies our previous study that the centrifugal drip is an ideal substrate to search for protein biomarkers potentially predictive of meat quality traits.

Acknowledgement

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