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ScienceDirect
Meat Science 77 (2007) 46–54
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Identification of pork quality parameters by proteomics

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Received 14 March 2007; received in revised form 13 April 2007; accepted 16 April 2007

Abstract

A major parameter for quality of pork is its waterholding capacity (WHC). Prediction of WHC immediately after slaughter would be of benefit both to slaughterhouses for reasons of better logistics and/or branding of premium-meat, and to consumers for improved quality of meat products such as ham.

In our pilot study on proteome analysis of porcine muscle by two-dimensional electrophoresis, we have identified at least three and possibly eight significantly changed proteins that may serve as marker proteins for waterholding capacity. The most clearly identified proteins are creatine phospho kinase M-type (CPK), desmin, and a transcription activator (SWI/SNF related matrix-associated actin-dependent regulator of chromatin subfamily A member1, SNF2L1). A possible mechanism of how these proteins may influence WHC is discussed. An optimised protocol for protein extraction that provides for sufficient amounts of relatively pure proteins has been developed. Further studies are needed to validate and extend our preliminary results.

Keywords: Meat quality; Marker proteins; Proteomics; Driploss

1. Introduction

Prediction of meat quality in the slaughter line on the day of slaughter is very much desired, from both a logistic and an economical point of view. The vast progress in the production of lean meat during the last decades has largely taken place at the expense of meat quality, causing serious problems in the tenderness and the waterholding capacity (drip loss) of meat. Drip loss may vary between 2% and 12%, which causes a direct economical loss because of reduced weight. Indirect losses are caused by reduced possibilities for processing of meat into high quality products such as ham. Rapid on-site prediction of meat quality, preferably even before the meat is chilled, may allow for allocation of carcasses to different production lines or for branding of meat (premium-meat, etc.). End-users, like meat/food processing companies, are becoming more aware that "raw" meat proteins must have consistent and specific quality and functionality characteristics. The latest consumer demands have already led to product differentiation and greater pressure on the value of meat quality parameters, especially tenderness, juiciness and flavor of fresh and value-added meat products (Sosnicki, Pommier, Klont, Newman, & Plastow, 2003). Moreover, tenderization through proteolytic degradation, and waterholding capacity are interrelated phenomena, as shown by a published model for possible causes of driploss (Kristensen & Purslow, 2001).

1.1. Tenderness

It is well established that meat tenderizes during storage; however, the underlying biochemical and physiochemical mechanisms during the tenderization processes are still a matter of dispute (Lametsch et al., 2003). In general, post-mortem degradation of muscle proteins is an important

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doi:10.1016/j.meatsci.2007.04.017

factor in the meat tenderization process (Koohmaraie, 1996; Hopkins & Thompson, 2002a), as postmortem degradation of several structural proteins including troponin T, nebulin, titin, vinculin, desmin and dystrophin has been demonstrated using one-dimensional SDS–PAGE and immuno-blotting (Hopkins & Thompson, 2002b). This is supported by the fact that titin, a giant muscle protein spanning from the Z-line to M-line region, and nebulin, which runs parallel with the thin filaments to the Z-line (Bandman & Zdanis, 1988), both have been shown to degrade faster in tender meat than in tough meat (Fritz & Greaser, 1991; Huff-Lonergan, Parrish, & Robson, 1995). Moreover, electron microscopy studies of the myofibrils during tenderization have shown that the attachment of the sarcolemma to myofibrils and the junctions between myofibrils at the level of the Z-disk and M-line are disrupted postmortem (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995), which suggests that proteolysis of the costamere proteins desmin, dystrophin, and vinculin, which attach the myofibrils to the sarcolemma, is taking place. Strong correlations have been found between postmortem degradation of troponin T and shear force (Huff-Lonergan et al., 1996; Steen, Claeys, Uytterhaegen, DeSmet, & Demeyer, 1997; Wheeler & Koohmaraie, 1999). However, whether the degradation of troponin T is an essential part of the tenderization process or just a marker for postmortem proteolytic activity is still unclear.

Proteome analysis by two-dimensional gel electrophoresis (2DE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) further extended the number of observed degrading proteins (in *M. longissimus dorsi* of pigs during the first 72 h postmortem) to 103, which included actin, myosin HC, myosin LC I and II, CapZ and cofilin (Lametsch et al., 2003). More recently, a tentative 2DE-based reference map encompassing 133 proteins was prepared by Hwang, Park, Kim, Cho, and Lee (2005).

Despite the fact that postmortem degradation of several structural proteins has been studied in great detail, it is still far from established whether any of these are directly responsible for the tenderization of meat.

The observation that desmin and vinculin are degraded with conditioning in bovine, porcine and chicken meat has generally led to the hypothesis, that meat tenderization involves degradation of intracellular cytoskeletal, i.e. intermediate filament (IF) structures. This may take place both by easing the separation of myofibrils and therefore weakening the lateral strength of meat, and by degradation of costameres which may weaken the sarcolemma, thereby increasing calcium influx and calpain activity (Purslow, Morrison, & Mielche, 1997).

1.2. Waterholding capacity

An accepted model of changes in waterholding capacity in muscle postmortem involves lateral shrinkage of myofibrils as the principle mechanism driving water loss (Kristensen & Purslow, 2001). Lateral shrinkage of myofibrils, muscle fibers and entire muscle fascicles, generates drip channels between fascicles. This mechanism requires that lateral connections formed by IFs through the costameres to extracellular structures are sufficiently strong to laterally displace fluid released by shrinkage of individual myofibrils to the extracellular spaces (Purslow et al., 1997).

It should be noted, however, that apart from involvement of IF there are many other factors, both genetic and non-genetic, that can influence waterholding capacity (Henckel, Karlsson, Oksbjerg, & Petersen, 2000). Newly discovered polymorph expression products in this respect are the melanocortin-4 receptor (Kim, Larsen, Short, Plastow, & Rothschild, 2000), the gamma3 subunit of AMP-activated proteinkinase (Milan et al., 2000), and the ryanodine receptor (Henckel et al., 2000). Non-genetic factors include stress and nutrition status of the animal before slaughter, creatine phosphate (CP) and glycogen content, ultimate pH and fibre type composition of meat, stunning method and cooling regime.

From a perspective of proteome analysis we consider it of importance, that IF-proteins seem to be implicated in two primary quality parameters of meat: tenderness and waterholding capacity.

1.3. Aim of this study

In this study we wanted to analyse the proteome of muscle tissue collected immediately after slaughter, and search for individual proteins that correlate with drip loss as measured at 5 days after slaughter. Identification of such (a) protein(s) could help predict the WHC of meat at a time that decisions in the slaughterhouse can be made, i.e. during the time period between killing of the animal and chilling of the carcass. A more general aim was the assessment of proteomics as a method for the identification of marker proteins that can predict meat quality.

2. Materials and methods

2.1. Animals and housing

It was hypothesized, that animals with little room for exercise may have less trained muscles and therefore impaired meatquality after slaughter, as compared to animals kept in a more stimulating housing system. By introducing two housing systems, ''enriched'' and ''barren'', we though it possible to induce extremes with respect to meatquality parameters, such as waterholding capacity.

Animals, housing system and experimental set-up were essentially as described earlier (Klont et al., 2001) except that the area per animal was 1.83 m^2 and 0.80 m^2 for the enriched and barren housing system, respectively, whereas in the previous study the difference was much smaller (1.16 m^2 and 0.84 m^2, respectively).

The experiment was set up in two replicates of 24 pigs. Twelve litters of a commercial crossbred line, equally

divided over the two different housing systems were used for selection of piglets. From each litter 2 piglets were selected (one male and one female), based on health and weight. Selection was made at 21 weeks of age, and piglets were allocated to their original housing system, i.e. either barren (half concrete area, half metal slats, no straw, 0.8 m^2/animal, 10 animals per pen) or enriched pens (daily fresh straw on concrete lying area, 0.7 m^2/animal indoor plus 1.13 m^2/animal outdoor, 8 animals per pen). All animals were fed ad lib.

2.2. Slaughter procedure and measurements

Animals were slaughtered at 25 weeks of age, and were deprived from food at the day before slaughter. Two replicates (group 1 and 2) were slaughtered on two different days, at a normal commercial slaughterhouse. After transport (1 h) to the slaughterhouse, pigs were kept in lairage for 2 h. Saliva samples were collected before transport and at the end of the lairage period, for the purpose of cortisol determination. Pigs were stunned electrically (300 V, 3 s) with a pair of stunning tongs, after which they were shackled by the hind leg and exsanguinated. Carcasses were chilled overnight at +4 oC. Muscle sampling and measurements were performed on 48 pigs. Temperature (Ebro TFN 1093 SK thermometer) and pH (Schott CG 818 pH meter with Xerolyt pH electrode) of the *Longissimus dorsi* (LD) and Biceps Femoris (BF) muscle were measured at 5 min, 45 min, 4 h and 24 h postmortem. Carcass weight was registered at 45 min postmortem. Carcass meat percentage and backfat thickness were determined with a Hennesy Grading Probe II at 45 min after slaughter.

Five minutes after exsanguination, samples were taken with a cork bore from the LD muscle at the height of the 3rd to 4th lumbar vertebra and from the BF muscle. Muscle cubes (0.5 cm^3) were cut from the LD muscle sample and the inside extreme end of the BF muscle cork bore sample. The BF sample represents the visually determined red portion of the BF muscle, whereas the LD sample represents a typically white muscle tissue. For BF it was considered that this muscle is especially active during walking, which makes it of interest with respect to the difference between the barren and enriched housing system.

All samples were rapidly frozen in solid carbon dioxide, and stored at −80 °C. From each cork bore sample, additional muscle cubes (0.5 cm^3) were cut and rapidly frozen in isopentane cooled with liquid nitrogen and stored at −80 °C, for the purpose of myofiber analysis and measurement of capillary density.

At 24 h postmortem some meat quality characteristics were determined on both LD and BF muscle samples. Water-holding capacity was measured according to the filter paper method of Kauffman et al. (Kauffman, Eikelenboom, Van der Wal, Merkus, & Zaar, 1986). Drip loss was determined as percentage of weight loss after 2 and 5 days storage at 4 °C according to Lundström and Malmfors (Lundström & Malmfors, 1985). Meat color was determined in triplicate after a 1-h blooming period by measuring L^*, a^*, and b^* values with a Minolta CM525i Chromometer.

2.3. Extraction of muscle protein

Pieces of 1 g of frozen muscle tissue were cut and weighed, and were immediately homogenized (Ultra Turrax homogenizer) in 12.5 mL cold pre-rigor extraction buffer of 50 mM Tris–HCL [Tris(hydroxymethyl)aminomethane–HCl], 10 mM EDTA (Ethylenediaminetetraacetic acid), 18 mM DTT (Dithiothreitol), pH 8.3] (Note: DTT was weighed and added immediately before extraction). The homogenate was divided into two equal parts for analysis of ''soluble'' and ''total'' protein, respectively. For ''soluble'' protein, the sample was centrifuged (3500 rpm, +4 °C, 15 min), the supernatant aliquotted and stored at −20 °C, and 100 μl used for protein purification (PlusOne 2D-Clean-up kit, Amersham Biosciences). For ''total'' protein, 300 μl homogenate was mixed with 1.2 mL of 10% SDS (Sodium dodecyl sulfate) and heated at 100 °C for 5 min. After centrifugation (Eppendorf centrifuge, 13,000 rpm, 10 min, rT), 100 μl of supernatant was used for protein purification (PlusOne 2D-Clean-up kit, Amersham Biosciences).

Losses of protein due to purification by Clean-up kit appeared to vary between 40% and 60%.

Precipitated proteins were finally dissolved in rehydration buffer of 8 M urea, 2% CHAPS [3-[(3-Cholamidopropyl)-dimethyl-ammonio]-1-propane sulfonate], 0.5% IPG buffer (ampholite-containing buffer for use with Immobiline DryStrip gels) w/v, 18 mM DTT.

After purification, samples were analysed for protein concentration by RC/DC Protein Assay Kit (BioRad).

Concentrations of purified protein in the soluble fractions of the low drip and the high drip composite samples were 690 and 484 μg per 450 μL, respectively. However, the samples have to be analysed in triplicate; for this reason and because of limitation of the volumes available, the amounts of protein per gel were 281 and 127 μg for the low drip and high drip group, respectively. With respect to the total protein fractions, the available amounts were large enough for highly sensitive silver-staining (Amersham), but not for MS-compatible silver-staining and subsequent MS analysis. In the underlying study, only soluble protein fractions were used for further detailed investigation.

For comparison of ''High Drip'' and ''Low Drip'' proteomes, purified samples of four selected animals with either high or low % drip loss were combined in such a way, that all animals contributed equal amounts of protein to the high drip or the low drip mixture, respectively.

2.4. Two-dimensional electrophoresis

Samples were analysed in triplicate.

First dimension separation of proteins on the basis of differences in isoelectric point (pI) was performed with Immobiline DryStrips (24 cm, pH 3–10, Amersham Pharmacia Biotech) and an IPGphor Isoelectric Focusing System, according to the instructions of the supplier. After isoelectric focusing and before 2nd dimension, the strips were equilibrated (15 min, rT) in 14 mL of SDS equilibration buffer [50 mM Tris–HCl pH 8.8, 6 M urea, 30% glycerol, 2% SDS and a few grains of BFB (Bromophenol Blue)] plus 140 mg DTT added immediately before use, followed by iodoacetamide equilibration (14 mL of SDS equilibrationbuffer plus 350 mg iodoacetamide added immediately before use).

Second dimension electrophoresis [MW separation by PAGE (Polyacrylamide gel electrophoresis)] was performed with an Ettan Dalt II System, and the use of Precast gels (12.5% polyacrylamide, $255 \times 196 \times 1$ mm, Amersham Biosciences).

Proteinstaining for spot detection was done with the highly sensitive Silver Staining Kit of Amersham Biosciences. For preparative purposes, i.e. excision of spots and protein analysis by mass spectrometry, gels were stained with the MS-compatible SilverQuest Silver Staining Kit of Invitrogen Life Technologies, according to a modified protocol.

Image analysis of 2D gels was performed with the PDQuest programme of BioRad.

Molecular weights were estimated by application of marker proteins (Low Molecular Weight SDS Calibration Kit for SDS electrophoresis, Amersham Pharmacia Biotech), and pI was estimated by assuming linear distribution of pI over the whole range of the Immobiline DryStrip pH 3–10. Randomly chosen proteins A B, C and D served as internal controls.

After 2D-elecrophoresis, which was followed by highly sensitive silverstaining (Amersham) and PDQuest analysis, approximately 700 spots per gel could be detected and were used for the alignment.

Statistical analysis was done by student *t*-test, as provided by the PDQuest software.

For the purpose of mass-spectrometry analysis, the experiment was repeated with different aliquots of the same composite samples but with maximal (i.e. three times more as compared to the Amersham method) protein concentrations, and proteins were stained with MS-compatible silver staining method (Invitrogen) After MS-compatible staining, each spot of a candidate marker protein was identified on the basis of previous PDQuest analysis, excised by placing a shortened pipet tip over it, transferred into an Eppendorff tube containing 100 μL of 10% methanol/1% acetic acid, and stored at −80 °C until analysis.

2.5. Chemicals and reagents; digestion of proteins from two-dimensional gels

Chemicals and reagents were of highest purity grade, preferably PlusOne-2D (Amersham Biosciences). Trypsin digestion and MS/MS analysis were essentially as described by Li et al. (2004).

The gel pieces were destained in 60% acetonitrile in 25 mM ammonium bicarbonate buffer, pH 8.5, and then dehydrated with 100% acetonitrile. The shrunken gel pieces were reswelled in 25 mM ammonium bicarbonate buffer, dehydrated again in 100% acetonitrile, and dried in a speedvac. For gel pieces that were heavily stained the rehydration/dehydration step was repeated once. The gel pieces were rehydrated in 8 μl of trypsin solution (20 μg/ml) for 1 h, followed by addition of 50 μl of 25 mM ammonium bicarbonate buffer to completely immerse the gel pieces. After incubation overnight at room temperature, 0.5 μl of the incubation buffer was pipetted to the MALDI plate and mixed with 1 μl of α-cyano-4-hydroxycinnamic acid. The samples were analyzed by a MALDI TOF/TOF® (Applied Biosystems) mass spectrometer (see below). In cases where the mass spectrometric signals were weak, the incubation buffer was loaded into a C18 Ziptip (Millipore) according to the protocol provided by the company. The bound peptides were eluted from the Ziptip using 1.0 μl of α-cyano-4-hydroxycinnamic acid, which was directly deposited onto the MALDI plate. The α-cyano-4-hydroxycinnamic acid matrix concentration was 5 mg/ml in 50% acetonitrile/50% water containing 0.1% trifluoroacetic acid.

2.6. Mass spectrometry of trypsin-digested protein spots from two-dimensional gels

The mass spectrometer utilized for the protein analysis was an Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF™ Optics. This MALDI tandem mass spectrometer uses a 200 Hz frequency-tripled neodinium YAG laser operating at a wavelength of 355 nm. For MS/MS, ions generated by the MALDI process were accelerated at 8 kV through a grid at 6.7 kV into a short, linear, field-free drift region. In this region the ions passed through a timed-ion selector (TIS) device that is able to select one peptide from a mixture of peptides at different m/z for subsequent fragmentation in the collision cell. After a peptide at a given m/z was selected by the TIS it passed through a retarding lens where the ions were decelerated and then passed into the collision cell, which was operated at 7 kV. The collision energy was defined by the potential difference between the source and the collision cell and hence was 1 kV. Inside the collision cell the selected peptide ions collided with air at a pressure of 1×10^{-6} Torr. After passing through the collision cell the ions (both intact peptide ion, the precursor, and fragments caused by collision with the air, the product ions) were reaccelerated in the second source region at 15 kV, passed through a second, field-free, linear drift region, into the reflector and finally to the detector. The detector amplified and converted the signal to electrical current, which was observed and manipulated on a PC-based operating system. For reflector mode the

operation of the instrument is far simpler. After the MALDI process generates the peptide ions they are accelerated at 20 kV through a grid at 14 kV into the first, short, linear, field-free drift region. After this point the rest of the instrument can be treated as a continuation of this field-free, drift region until the ions enter the reflector and then reach the detector where, as before, the signal at the detector is amplified and converted to electrical current.

2.7. Search criteria

The MS resolution for the peptides was generally greater than 10.000 full-width half-maximum, and the mass accuracy better than 10 ppm. For the data base search the mass tolerance was set at 0.03 Da. The MS/MS resolution was ~3000–6000 full-width half-maximum. No internal standard was used for calibration and the mass tolerance was set higher at 0.3 Da although mass error was expected to be less than 0.1 Da. All the mass spectra were searched against the NCBI data base, using online Mascot software (Matrix Science), to identify the proteins.

The identification of the proteins is based on several criteria. The most important criterion is the mascot score of the peptide mass fingerprint (PMF), and in most cases the scores are significant. Further, the mascot scores of the daughter ion spectra of the tryptic peptides were also considered, as well as the matching of the identified proteins to the calculated M_r and pI.

3. Results

3.1. Selection of samples for 2D-analysis

Drip loss was considered one of the most important meat quality parameters of pork. For this reason, animals were ranked according to increasing drip loss both for BF

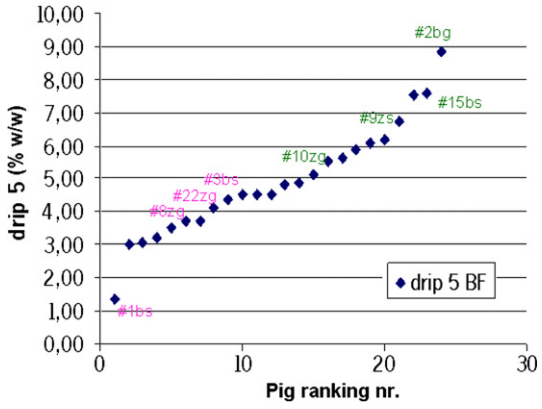

Fig. 1. Ranking of 24 animals according to % drip loss of BF (Biceps Femoris) muscle. Animals 2, 9, 10 and 15 were selected for high % drip loss, and animals 1, 3, 8 and 22 were selected for low % drip loss. (drip 5 = % drip loss measured at day 5 postmortem). z = sow, b = barrow, s = enriched, g = barren.

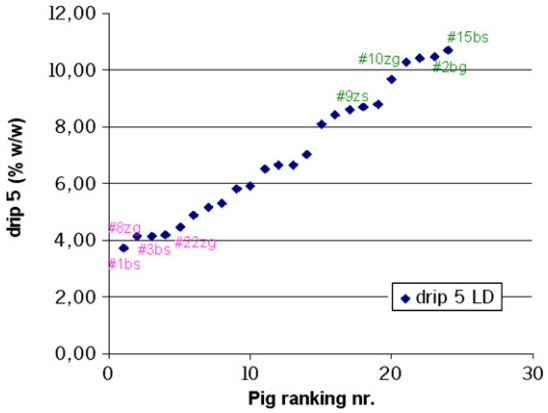

Fig. 2. Ranking of 24 animals according to % drip loss of LD (*longissimus dorsi*) muscle. Numbers and indices as in Fig. 1. The same animals were selected both for LD and BF (Fig.1).

(Fig. 1) and LD (Fig. 2), and 4 animals were selected either with high or with low % drip loss in both muscle types. Selection was made in such a way, that animals were equally divided over sex and housing system, and no littermates were allocated to the same group (with one exception: animals #9 and #15 were from the same litter).

It is well known that extreme values of ultimate pH (at 24 h postmortem) can result in abnormal meat quality characteristics. For this reason, selected animals were checked for abnormal ultimate pH in both BF and LD. It appeared that the ranges of ultimate pH values for BF in the low drip and high drip group were 5.65–5.87 and 5.53–5.73, respectively; for LD the ranges of ultimate pH in the two groups were 5.49–5.65 and 5.43–5.51, respectively. These values were considered to be normal.

3.2. Candidate marker proteins

Comparison of the high drip and low drip groups showed that 8 spots were consistently different (Fig. 3).

It appeared that only 3 out of selected 8 spots contained enough protein to allow for identification by MS/MS analysis. Clear identification by MS was observed for candidates 2, 7 and 4. Candidate 2 was found to be creatine kinase M-type; calculation of MW and pI further confirmed its identity. For candidate 7, confirmation of its identity as desmin was obtained from data on MW and pI. However for candidate 4 (SWN/SNF-related matrix-associated actin-dependent regulator of chromatin a 1 isoform b) the calculated pI or MW were different from the theoretical values as obtained for either rat or human (Table 1). Nevertheless it was found that candidate 4 is a protein that is partly homologous to human SNF2L1 transcription activator. The SNF2L1 transcription activator is functionally linked to ATP (Adenosine triphosphate)-binding and actin-dependency, and therefore it can be hypothetically linked in some (as yet unknown) way to postmortem intra muscular pH and waterholding capacity.

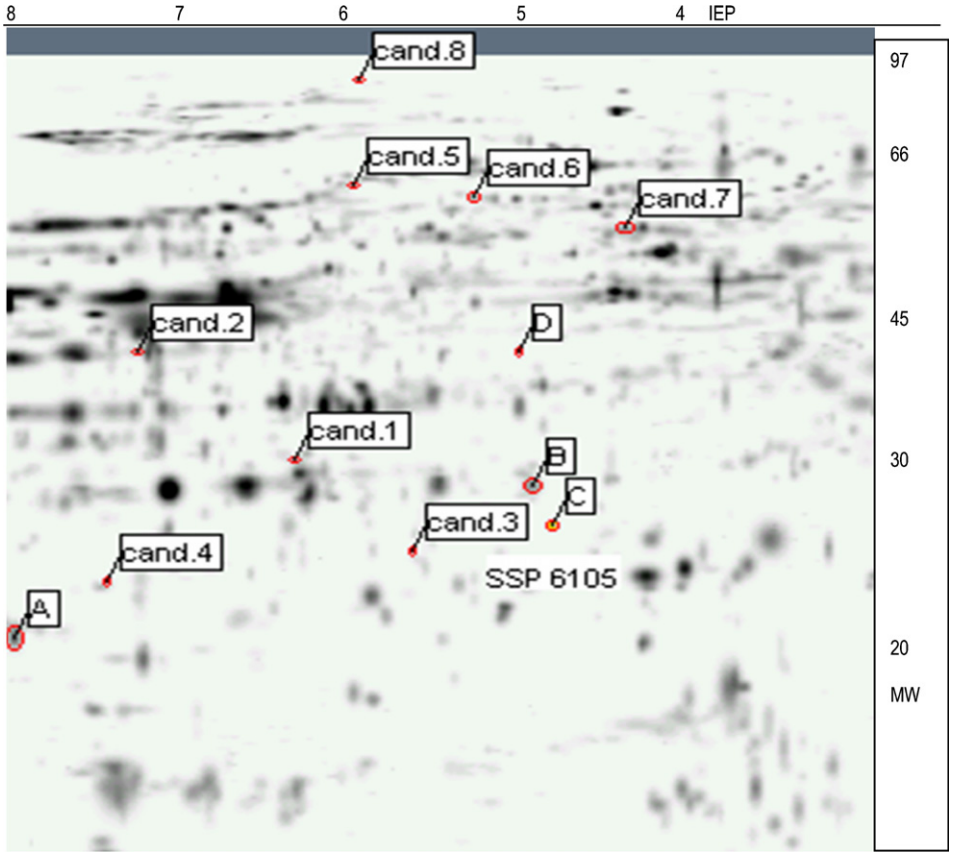

Fig. 3. Candidate marker proteins (no's 1–8) in the Matchset according to PDQuest analysis. Candidates 1–6 are elevated in the high drip sample, and candidates 7–8 are elevated in the low drip sample. A, B, C and D are randomly chosen spots ranging in density from very strong to very weak, in order to serve as internal controls. MW = Molecular weight, IEP = Isoelectric point.

Table 1
Protein identification by MS/MS, MW and pI. Sequence coverage (SC, %) and number of matching peptides are also given

cand/spotnr	MS/MS MALDI-ToF mass fingerprint	MW estimated	MW theoretical	pI estimated	pI theoretical	NCBI gi no.	SC (%)	Number of matching peptides	Reference species
cand2/1303	Creatine kinase	43,652	43,059	7.20	6.61	gi/17979615	74	9	Porcine
cand7/7607	Desmin	58,884	53,497	5.16	5.21	gi/2959454	29	10	Porcine
cand4/1011	*SWI/SNF-related matrix-associated actin-dependent regulator of chromatin a1, isoform a	26,915	123,514	7.30	8.67	gi/27709746	16	17	Human rat
	*id. isoform b SNF2-like 1; Global transcription activator homologous sequence	26,915	120,231	7.30	7.79	gi/21071046	16	17	Human

4. Discussion and conclusions

4.1. Candidate marker proteins

Although the observation of creatine kinase as a candidate marker protein for waterholding capacity still needs confirmation, it certainly makes sense in the total picture of muscle physiology and its relation to meat quality. Creatine kinase is known for enzymatic conversion of creatine phosphate into creatine and ATP (Fig. 4). The conversion of muscle to meat is an energy-demanding process and in the muscle after death, as well as in life, the energy is provided by splitting of ATP to ADP and inorganic phosphorus (Henckel, Karlsson, Jensen, Oksbjerg, & Petersen, 2002). In the muscle after death, the ATP is replenished by the conversion of ADP to ATP by the transfer of the higher energy phosphate for creatine phosphate and by degradation of glycogen. The observed decline in pH depends on this ability by the formation of lactate from the available glycogen (Bendall, 1973).The basic biochemical reactions underlying the pH decline postmortem and how this decline exerts a strong influence on a number of important meat

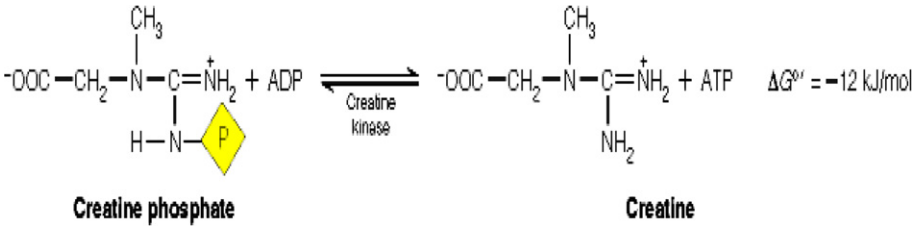

Fig. 4. The reaction is strongly endergonic as written. However, the level of ATP is very high in mitochondria, so the reaction proceeds to the left. Creatine phosphate then diffuses from mitochondria to the myofibrils, where it provides the energy for muscle contraction.

quality characteristics are well recognised (Bendall, 1973; Bendall, 1951). In short, creatine phosphate (CP) is firstly degraded. When this is reduced to approximately 25% of its resting value, a decrease in glycogen and a concomitant decrease in ATP are observed. The period, during which CP is still present in sufficient amounts, is referred to as the delay phase by analogy to the onset of rigor (Bendall, 1973). The decline in pH thus depends on the initial concentration of creatine phosphate and glycogen (Bendall, 1951), and in practical situations large variations in the course of pH decline can be expected (Henckel et al., 2002), which may relate to variations in drip loss and meat quality.

The enzyme creatine phospho kinase (CPK), which is responsible for conversion of creatine phosphate into creatine and ATP, is located in the myofibrils particularly in the M-line (for a review: see Schreurs, 1999). The M-line is the place in the sarcomere where thick or myosin filaments are kept in lateral register both transversely and longitudinally. Myosin together with actin belongs to the socalled contractile proteins, which are responsible for the mechanical contraction of the muscle. Therefore CPK may have an effect on the rate of muscle contraction postmortem, by its effect on CP degradation, rate of decline of glycogen and concomitant change of pH. Our observation that CPK levels are higher in muscle with high drip loss may very well fit in our drip loss hypothesis, in a way that high CPK levels cause shortening of the delay phase by rapidly degrading creatine phosphate. This may in turn cause more rapid pH decline and muscle contraction, and therefore result in high drip loss.

Another potential marker protein as identified by our study is desmin. Desmin belongs to the group of cytoskeletal proteins, which are responsible for integrity and rigidity of the muscle cell. Desmin is located in the intermediate filaments; it is responsible for the lateral structure and integrity of the myofiber because it holds adjacent myofibrils together at the Z-line level. It is generally accepted that the source of drip from pork is intracellular water which is lost from the muscle fibre postmortem, driven by a pH and calcium-induced shrinkage of myofibrils during rigor development (Honikel, Kim, & Hamm, 1986; Offer et al., 1989). The rate and quantity of drip formation in fresh meat is believed to be influenced by the extent of rigor-shrinkage and the permeability of the cell membrane to water as well as other factors, such as the extent of protein denaturation. It has also been recognised that an intact cytoskeleton within the muscle is necessary to translate shrinkage of myofibrils into shrinkage of the whole cell (Offer & Knight, 1989). Adjacent myofibrils are connected by desmin-rich intermediate filaments and vinculin-rich sarcomeric structures connect peripheral myofibrils to the sarcolemma (Stromer, 1998). It is hypothesised that a lateral shrinkage of laterally connected myofibrils results in a shrinkage of the whole muscle fibre and thereby squeezes out water (Offer & Knight, 1989). The water accumulates extra-cellularly between muscle fibre bundles and at a later stage also between single muscle fibres. From these extracellular compartments water slowly drains to the surface where it forms drip (Offer & Cousins, 1992).

Proteolytic degradation of desmin in rapidly aging muscles has been demonstrated during the period 24–72 hrs postmortem (Christensen, Henckel, & Purslow, 2004). The rate of desmin degradation may therefore be related to the duration and degree of myofibril shrinkage and therefore to the phenomenon of drip loss. On the other hand, in a recent 2DE-study on postmortem proteolysis no relationship was found between desmin and driploss (Hwang et al., 2005). This may be partly explained by the fact that protein identification for aged muscle (i.e. at 3d) proved extremely complicated, whereas in our study we analysed samples that were taken immediately after slaughter. Desmin clearly fits in the currently accepted model for muscle contraction and WHC (Kristensen & Purslow, 2001) and therefore is very likely to be a marker protein for drip loss. Our observation that desmin levels are higher in muscle with low drip loss still has to be reconciled with our mechanistic model for drip loss.

4.2. Possible practical applications

For a slaughterhouse it is important to have information at a very early stage, i.e. immediately after slaughter, about the expected quality of the meat. For this purpose it might be feasible to develop a protein microarray, that can quantify individual marker proteins or give discriminatory binding patterns of combined marker proteins. Such a protein microarray may have the potential advantage over DNA microarrays, that its measurements are much closer to the factor of interest, i.e. meat quality.

Development of dipstick tests for rapid on-line testing could be another option.

Furthermore, identification of marker proteins for drip loss can fortify hypothetical models on the mechanism of drip loss, and can possibly result in preventive measures. It can also prompt further studies into their corresponding genes, because the possibility of polymorphisms may allow for genetic selection against drip loss. This can certainly be of value for commercial breeding companies, who seek to improve meat quality characteristics of their breeding stock.

5. Conclusion

It is concluded that proteomics analysis can be a successful method to identify marker proteins for prediction of meat quality. In our study, candidate marker proteins have been identified that are highly relevant to the process of driploss. Continued research almost certainly will lead to additional marker proteins being identified.

Acknowledgements

The authors wish to thank Mr. Roel van der Schors and Dr. Ka Wan Li, Department of Molecular and Cellular Neurobiology, Research Institute Neurosciences, Faculty of Earth and Life Sciences, Vrije Universiteit, Amsterdam, for MS/MS analysis. They also wish to thank Dr. Jelle Thole and Mrs. José Westerveen of Animal Sciences Group of Wageningen UR for their guidance in 2D-electrophoresis.

One of the authors (Z.W.) gratefully acknowledges a LNV-IAC fellowship from the Dutch Ministry of Agriculture, Nature and Foodquality.

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