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Proteomic and peptidomic differences and similarities between four muscle types from New Zealand raised Angus steers

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ABSTRACT

Four muscles from New Zealand-raised Angus steers were evaluated (*musculus semitendinosus*, *m. longissimus thoracis et lumborum*, *m. psoas major* and *m. infraspinatus*) to test their differences and common features in protein and peptide abundances. The ultimate goal of such a comparison is to match muscle types to products with targeted properties.

Protein profiling based on two-dimensional electrophoresis showed that the overall profiles were similar, but, between muscle types, significant ($p < 0.05$) intensity differences were observed in twenty four protein spots.

Profiling of endogenous peptides allowed characterisation of 346 peptides. Quantitative analysis showed a clear distinction between the muscle types. Forty-four peptides were identified that showed a statistically significant ($p < 0.05$) and substantial (>2-fold change) difference between at least two muscle types.

These analyses demonstrate substantial similarities between these four muscle types, but also clear distinctions in their profiles; specifically a 25% difference between at least two muscles at the peptidomic level, and a 14% difference at the proteomic level.

1. Introduction

More than 12% of New Zealand's total export income relies on meat and meat products (beef, sheep and deer), annually contributing over $5.6B to the New Zealand GDP (Statistics New Zealand, 2014). Boosting opportunities for onshore value addition in food products and increasing the value of meat through quality improvement are key economic drivers for the NZ food industry. To a large extent, future value addition is expected to be dependent on understanding and managing the underpinning key quality attributes of primary meat components.

Flavour, colour, texture and nutritional value comprise the most essential quality attributes for all varieties of meat and meat products, with a direct correlation to value and international competitiveness. Meat consists of 10–30% proteins and 5–35% lipids, depending on the species and the cut (Paul & Southgate, 1985). Meat flavour derives from its profile of proteins, peptides, carbohydrates and lipids (Spanier et al., 2004; Wood et al., 1999). Proteolysis and lipolysis are critically involved in the development of the flavour of meat products, while lipids and their oxidation products particularly influence species-specific flavour. An important contributor towards meat colour is the chemistry of myoglobin and other associated proteins (Kerry & Ledward, 2009; Mancini & Hunt, 2005). Additional factors influencing colour include lipid oxidation, which has been correlated with meat discolouration and the formation of proteinaceous chromophores and fluorophores (Erickson, 1997; Mancini & Hunt, 2005). The texture of meat is directly related to its protein matrix, and is affected by post-translational changes such as glycosylation, protein backbone cleavage, aggregation, oxidation and crosslinking (Nishimura, 2010; Lund, Heinonen, Baron, & Estévez, 2011). In terms of nutritional value, proteins and lipids represent the key macronutrients in meat; protein–protein and protein–lipid crosslinks, protein cleavage and amino acid damage are associated with deterioration of nutritional value (Erickson, 1997; Love & Pearson, 1971).

Correlating meat quality attributes with biomolecular composition (notably proteins and lipids) is a complex problem because of the

Abbreviations: 2D-DIGE, two-dimensional difference gel electrophoresis; LC, liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CID, collision-induced dissociation; ETD, electron transfer dissociation; Q-TOF, quadrupole time-of-flight.

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many variables that have to be considered, including animal breed, feeding regime, muscle type, protein/peptide/lipid profiles, meat ageing, and consumer preferences. This type of exercise is often referred to as 'advanced phenotyping'. Its aim is to understand precise links between industry-relevant phenotypes (e.g., tenderness, fat colour and marbling) and their causative components. It has the potential to attribute a new level of control over product parameters, e.g., allowing enhancements in the conversion of muscle to meat, or leading to a predictive model to correlate protein/lipid patterns with specific traits. Another example of active research in this field is the examination of the relationship between meat quality traits and post-mortem protein profile changes (Gobert, Sayd, Gatellier, & Santé-Lhoutellier, 2014; Paredi et al., 2013; Wu, Farouk, Clerens, & Rosenvold, 2014).

A necessary step towards advanced phenotyping is cataloguing and understanding biomolecule profile differences between muscles, because it is expected that the different biomolecular composition of a given muscle will determine the functionality and use of that muscle for particular applications. For example, if certain muscles are shown to have a high concentration of a particular protein, they could be preferentially targeted for certain niche markets or for development of higher value derived products; or if a particular bioactive peptide is particularly abundant in a certain muscle at a defined point during ageing, this can be targeted for extraction of functional ingredients.

Protein and peptide profiles in muscle are in a state of change from the time of slaughter, during meat ageing, storage, retail display and through to preparation, consumption and digestion. It is recognised that there are many points of significant interest for biochemical analysis along that timeline, and many studies have been devoted to determining protein profiles in muscles (Jia, Hollung, Therkildsen, Hildrum, & Bendixen, 2006; Joseph, Suman, Rentfrow, Li, & Beach, 2012; Oe et al., 2011), protein breakdown during ageing (Huff Lonergan, Zhang, & Lonergan, 2010), changes induced by cooking (Deb-Choudhury et al., 2014; Sarah, Karsani, Amin, Mokhtar, & Sazili, 2014) and peptide release during digestion (Kaur, Maudens, Haisman, Boland, & Singh, 2014; Wen et al., 2014). For the present study, we decided to conduct comparisons between four key muscles (*musculus semitendinosus* (ST), *m. longissimus thoracis et lumborum* (LTL), *m. psoas major* (PM) and *m. infraspinatus* (IS)) from New Zealand-raised Angus steers, sampled at a very early stage in the meat production value chain. Specifically, we wanted to investigate these muscles in relation to their potential use as starting materials for high value functional ingredients or production of meat-derived products, and therefore test and understand any points of difference and similarity between them in protein and peptide abundances.

2. Materials and methods

2.1. Meat sampling and storage

Ten Angus steers, 34 months old, grass-fed and finished on grain for 130–140 days, were randomly selected for sampling in a local abattoir (Canterbury Meat Packers, Ashburton, New Zealand). The animals were stunned across the head (neck to nose for 3 s at 400–500 V at 2 A) after which the current was passed from neck to brisket (for 14 s of 450–450 V at 2.5 A). The steers then exited the stun box and were electrically stimulated during bleeding by application of 80 V peak, 14.28 pulses s^{-1} for 30 s. Muscle pH measurements were as follows: 5.48; 5.42; 5.54; 5.46; 5.48; 5.48; 5.43; 5.46; 5.48; and 5.45. Carcass weights were as follows: 181 kg; 205.5 kg; 229.5 kg; 234 kg; 214.5 kg; 238 kg; 210 kg; 273.5 kg; 255 kg; and 275 kg.

A muscle sample, approximately 20 × 20 × 50 mm, was taken 20 min post-mortem, from the following four muscles: ST, LTL, PM and IS.

The samples were snap frozen on solid CO_2 and stored at −80 °C. Further analysis was performed after 3.5 years.

2.2. Protein extraction

Pooled samples of each muscle type were prepared by combining 100 mg of tissue from each of the ten animals. Proteins were extracted by homogenising 500 mg of these samples in 5 ml of lysis buffer containing 7 M urea, 2 M thiourea, 1% dithiothreitol, pH 8.5 for 1 min using a Teflon homogeniser, keeping the sample in an ice bath. After vortexing the homogenate for 30 min at 4 °C, the insoluble material was pelleted by centrifugation of the homogenate at 15,000 rpm for 30 min at 4 °C. The pellet was removed and the supernatant was collected. The protein concentrations were determined using the 2D-Quant kit according to the manufacturer's instructions (GE Healthcare, USA). Protein extracts were further analysed using two-dimensional difference gel electrophoresis (2D-DIGE) analysis.

2.3. Peptide solubilisation

Pooled samples of each muscle type were prepared by combining 100 mg of tissue from each of the ten animals. Peptides were extracted by homogenising 500 mg of these samples in 0.25% acetic acid containing 2% acetonitrile, vortexing for 30 min at 4 °C, and centrifuging at 20,000 rpm for 30 min at 4 °C. The supernatant was diluted 3× in 0.25% acetic acid containing 2% acetonitrile, and 400 μl was passed through a NanoSep 10 K Omega centrifugal filter (Pall, Hamilton, New Zealand). Peptide concentrations were measured by infrared absorption using a Direct Detect instrument (Merck Millipore, Mairangi Bay, New Zealand) according to the manufacturer's instructions. After that, the peptides were analysed using LC–MS and LC–MS/MS. Peptide concentrations were as follows: IS 12.3 mg/ml; ST 10.4 mg/ml; LTL 13.3 mg/ml; PM 11.9 mg/ml.

2.4. 2D-DIGE

Prior to protein labelling, the pH of the sample was measured and, if necessary, adjusted to 8.5 using 100 mM sodium hydroxide. An internal protein standard was prepared from a mixture of equal amounts of all the samples used in the experiment, to allow comparison across experiments.

The protein samples were labelled with Cy3 or Cy5 cyanine dye and the internal standard with Cy2 dye, by adding 400 pmol of CyDye per 50 μg protein. After labelling, samples were incubated on ice for 30 min in the dark. The reaction was quenched by adding 1 μl of 10 mM L-lysine (Sigma, St. Louis, MO, USA) solution followed by incubation for 10 min on ice in the dark.

After labelling, the samples were combined as shown in Table 1 and each mixture, containing 150 μg protein, was diluted with rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 1.2% DeStreak, 2% v/v isoelectric focusing buffer pH 3–10, 50 mM dithiothreitol and 0.002% bromophenol blue) to a final volume of 450 μl. The labelled sample mixtures were then applied to IPG strips (pH 3–11 NL, 24 cm), rehydrated overnight and proteins were subsequently focused at 100 V for 1 h, 500 V for 1 h, 1000 V for 2 h in a Protean IEF Cell (Bio—Rad Laboratories, Hercules, CA, USA). Focusing continued at 5000 V until 70,000 V h were reached.

Prior to the second dimension separation, the IPG strips were reduced for 15 min with 1.5% dithiothreitol and alkylated with 2.5%

Table 1
Gels and labelling scheme.

Gel	Cy2	Cy3	Cy5
1	Internal Standard	ST	IS
2	Internal Standard	IS	ST
3	Internal Standard	ST	IS
4	Internal Standard	PM	LTL
5	Internal Standard	LTL	PM
6	Internal Standard	PM	LTL