# Determination of Meat Authenticity Using a Comprehensive Targeted Proteomic Strategy and High-Resolution Mass Spectrometry

## Highlights

- Sensitive and robust HRAM LC-MS method using a Thermo Scientific™ Q Exactive™ mass spectrometer for the identification and detection of marker proteins in raw meat samples
- Four proteotypic peptides were used to perform accurate meat speciation and authenticity
- Myoglobin tryptic peptides from each species were detected with an observed *m/z* below 1.3 ppm
- Targeted method allowed for the detection of undesired meat species down to 1% (w/w) of the entire sample
- Data-independent acquisition (DIA) method can be used to detect all four proteotypic peptides

## Introduction

Due to the internationalization of food production and distribution, there has been a significant increase of food fraud in recent years. Food fraud can have serious health implications and occurs when food manufacturers implement unethical practices, such as making false label claims as well as using additives and fillers within their products to increase profitability. This has been a serious concern, and in 2013, horse and pig DNAs were detected in beef products sold by several retailers (DG Health and Consumers, European Commission). In an effort to control this within the food industry, certification of meat authenticity must be delineated for all regulatory agencies.

The application of proteomics in the meat science field is focused on improving meat quality while increasing meat production and revenue. To ensure that food safety regulations are being met, food-testing laboratories require more advanced analytical strategies to test for adulteration and to expose many of these unethical, albeit profit-generating, tactics. Since mass spectrometry (MS) is considered a gold standard in protein research, it is also used as a method for detecting marker proteins that support animal tissue identification. In this application, meat adulteration was tested using a well-defined proteogenomic annotation and carefully selected surrogate tryptic peptides. This novel method is a new technique for determining meat authenticity and composition using a state-of-the-art high-resolution Orbitrap™ MS.

## Experimental

### Sample Preparation

In order to verify assay sensitivity and specificity, raw pork meat was mixed at several weight percentage ratios (1, 2, 10, 25, 50, and 100) with a mixture of equal weight (1:1:1) raw beef:horse:lamb meat. All mixtures were performed to yield a total weight of 100 g. From these mixtures, 1 g was combined with 5 mL of distilled water, homogenized, and used for analysis.

Proteins were extracted and then digested with 2 µg of proteomic-grade trypsin at 40 °C for 24 h. Protein digestion was terminated by adding 500 µL of a 1% TFA solution. Afterwards, the samples were centrifuged at 12,000 g for 10 min, and 200 µL of the supernatants were transferred to injection vials for LC-MS analysis. Protein identification was performed using Thermo Scientific™ Proteome Discoverer™ software.

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## MS Conditions

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| MS: | Thermo Scientific Q Exactive benchtop quadrupole-Orbitrap mass spectrometer |
| Scan Type: | Full scan MS |
| Resolving Power: | 140,000 (FWHM) |
| AGC: | $3.0 \times 10^6$ |
| Maximum IT: | 200 ms |
| Scan Range: | $m/z$ 500–2000 |
| njection Volume: | 2 µL |
| Spray Voltage: | 4 kV |
| Capillary Temperature: | 300 °C |
| Sheath Gas Flow Rate: | 10 Arb |
| Auxilliary Gas Flow Rate: | 5 Arb |

*Product Ion Spectra Obtained with:*

| | |
|---|---|
| Resolving Power: | 17,500 (FWHM) |
| Collision Energy: | 25 |
| AGC: | $1.0 \times 10^{-6}$ |
| Maximum IT: | 100 ms |
| Isolation Window: | 1.5 Da |

## HPLC Conditions

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|---|---|
| System: | Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system |
| Column: | Thermo Scientific™ BioBasic™ C8 (5 µm, 100 × 1 mm) |
| Mobile Phases: | (A) water + 0.1% formic acid (B) acetonitrile + 0.1% formic acid |
| Inj. Volume: | 2 µL |
| Flow Rate: | 75 µL/min |

## Data

Table 1. Specific myoglobin proteotypic peptides for selected mammalian meat species.

| Species | Tryptic Peptide Sequence MB (120–134) | Theoretical Mass ($z = 2$) | Observed Mass ($z = 2$) | Mass Accuracy (ppm) |
|---|---|---|---|---|
| Beef | HPSDFGADAQAAMSK | 766.8435 | 766.8436 | 0.13 |
| Horse | HPGDFGADAQGAMTK | 751.8383 | 751.8378 | −0.67 |
| Pork | HPGDFGADAQGAMSK | 744.8304 | 744.8314 | 1.34 |
| Lamb | HPSDFGADAQGAMSK | 759.8357 | 759.8363 | 0.79 |

Table 2. MS/MS parameters used for the acquisition of myoglobin proteotypic peptides (MB 120–134).

| Species | Targeted Peptide | Precursor Ion Mass ($z = 2$) | Isolation Width (Da) | Collision Energy | Product Ion $m/z$ ($z = 1$) |
|---|---|---|---|---|---|
| Beef | HPSDFGADAQAAMSK | 766.8 | 1.5 | 25 | 1298.5681 (y13) 1395.6209 (y14) |
| Horse | HPGDFGADAQGAMTK | 751.8 | 1.5 | 25 | 1268.5576 (y13) 1365.6103 (y14 |
| Pork | HPGDFGADAQGAMSK | 744.8 | 1.5 | 25 | 1254.5419 (y13) 1351.5957 (y14) |
| Lamb | HPSDFGADAQGAMSK | 759.8 | 1.5 | 25 | 1285.5525 (y13) 1381.6053 (y14) |

Table 3. Other specific proteotypic peptides identified for selected mammalian meat species.

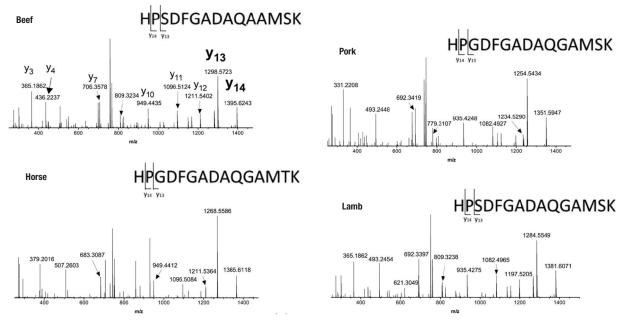| Species | Protein | Uniprot Accession Number | Peptide Sequence | AA Position | Theoretical Mass ($z = 2$) | Observed Mass ($z = 2$) | Mass Accuracy (ppm) | $R_t$ (min) |
|---|---|---|---|---|---|---|---|---|
| Beef | Myosin-1 | Q9BE40 | TLALLFSGPASGEAEGGPK | 619–637 | 901.4702 | 901.4694 | −0.89 | 16.8 |
| Horse | Myosin-1 | Q8MJV0 T | LALLFSGPASADAEAGGK | 619–637 | 888.4623 | 888.4620 | −0.34 | 17.0 |
| Pork | Myosin-1 | Q9TV61 | TLAFLFTGAAGADAEAGGGK | 619–638 | 912.9600 | 912.9594 | −0.66 | 17.4 |
| Lamb | Myosin-1 | XM_004012706.1 (RefSeq) | TLAFLFSGAASAEAEGGGAK | 619–638 | 927.9652 | 927.9650 | −0.21 | 17.6 |
| Beef | Myosin-2 | Q9BE41 | TLAFLFSGTPTGDSEASGGTK | 619–639 | 1022.4971 | 1022.4968 | −0.29 | 16.4 |
| Horse | Myosin-2 | Q8MJV1 | TLALLFSGAQTADAEAGGVK | 617–636 | 960.5073 | 960.5070 | −0.31 | 17.0 |
| Pork | Myosin-2 | Q9TV63 | TLAFLFSGAQ TGEAEAGGTK | 619–638 | 978.4891 | 978.4894 | −0.31 | 17.1 |
| Lamb | Myosin-2 | XM_004012707.1 (RefSeq) | TLALLFSGTPTAESEGSGTK | 617–636 | 984.0020 | 984.0022 | 0.20 | 16.5 |
| Beef | ß-Haemoglobin | P02070 | FFESFGDLSTADAVMNNPK | 40–58 | 1045.4804 | 1045.4796 | −0.77 | 16.9 |
| Horse | ß-Haemoglobin | P02062 | FFDSFGDLSNPGAVMGNPK | 42–60 | 1000.4646 | 1000.4637 | −0.90 | 17.2 |
| Pork | ß-Haemoglobin | P02067 | FFESFGDLSNADAVMGNPK | 2–60 | 1023.4673 | 1023.4670 | −0.29 | 16.8 |
| Lamb | ß-Haemoglobin | P02075 | FFEHFGDLSNADAVMNNPK | 40–58 | 1076.9915 | 1076.9906 | −0.84 | 15.3 |

Figure 1. Product ion spectra of myoglobin proteotypic peptides (120–134). Fragment ion selectivity is preserved for y14 and y13 ions. These products can be used to produce specific product ion XICs for meat speciation.
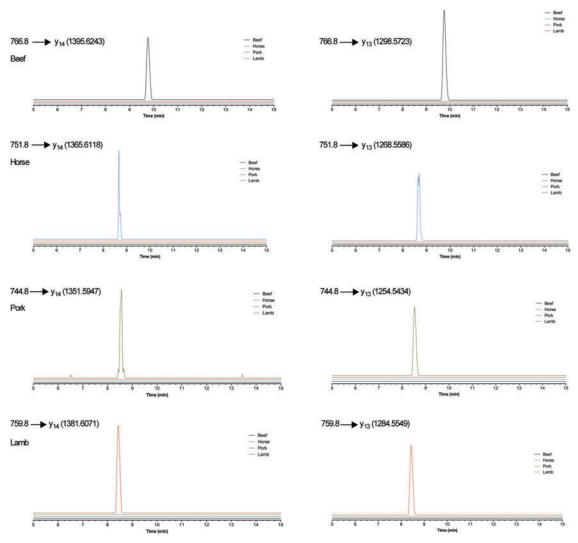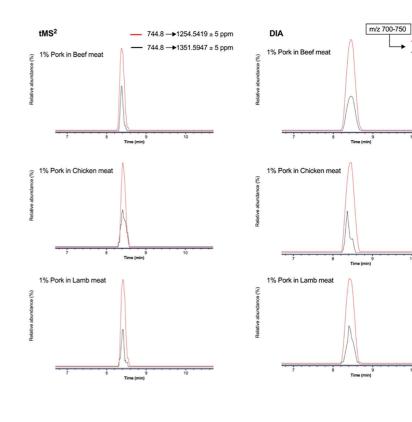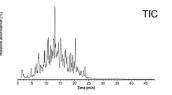


Figure 2. Extracted ion chromatograms for specific signature proteotypic peptide-fragment pairs. The data shows that each selected peptide-fragment pair was highly specific, which allowed it to perform accurate meat speciation. Additionally, very similar results were obtained using peptide precursor ion ($z = 2$) ± 5 ppm extracted ion chromatograms (data not shown).

Figure 3A. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide-fragment pairs. Chromatograms from meat samples spiked with 1% pork meat. (Extracted blank chromatograms are in blue.)



Figure 3B. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide-fragment pairs. Chromatograms from beef samples spiked with 1% horse meat. (Extracted blank chromatograms are in blue.)

## Conclusion

Muscular proteins from raw meat samples were methodically analyzed in silico to generate tryptic peptide mass lists and theoretical MS/MS spectra. Bottom-up proteomic analysis was utilized to detect and identify a proteotypic myoglobin tryptic peptide for each species with an observed $m/z$ below 1.3 ppm. Proteotypic peptides were also identified from myosin-1, myosin-2, and ß-haemoglobin. This targeted method allowed for the detection of undesired meat species down to 1% (w/w) of the entire sample with a potential to go significantly lower using straightforward sample enrichment techniques.

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