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

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ABSTRACT

Purpose: Describe a sensitive, robust HRAM LC-MS method to detect and identify marker proteins in raw meat samples,

Methods: Well-defined proteogenomic annotation, carefully selected surrogate tryptic peptides, and analysis using a hybrid quadrupole-Orbitrap™ MS were integrated.

Results: The targeted method allowed for the detection of undesired meat species down to 1% (w/w) of the entire sample. Myoglobin tryptic peptides from each species were detected with an observed *m*/*z* below 1.3 ppm.

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.

MATERIALS AND METHODS

Sample Preparation

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 ºC 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.

Test Method

HPLC analysis was performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC system equipped with a Thermo Scientific™ BioBasic™ C8 (5 µm, 100 × 1 mm) column. Mobile phases were (A) water + 0.1% formic acid, and (B) acetonitrile + 0.1% formic acid. The injection volume was 2 µL and the flow rate was 75 µL/min.

MS analysis was performed using a Thermo Scientific™ Q Exactive™ benchtop quadrupole-Orbitrap mass spectrometer.

Table 1. General MS conditions and specific conditions used for the acquisition of product ion spectra.

MS Conditions	
Scan Type	Full Scan MS
Resolving Power	140,000 (FWHM)
AGC	3.0×10^6
Maximum IT	200 ms
Scan Range	*m*/*z* 500–2000
Spray Voltage	4 kV
Capillary Temperature	300 ºC
Sheath Gas Flow Rate	10 Arb
Auxiliary Gas Flow Rate	5 Arb
Product Ion Spectra Obtained With:	
Resolving Power	17,500 (FWHM)
Collision Energy	25 eV
AGC	1.0×10^6
Maximum IT	100 ms
Isolation Window	1.5 Da

Data Analysis

Protein identification was performed using Thermo Scientific™ Proteome Discoverer™ software.

RESULTS

Table 2. 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 3. 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 (eV)	Product Ion *m*/*z* (z = 1)
Beef	HPSDFGADAQAAMSK	766.8	1.5	0.13	1298.5681 (y13) 1395.6209 (y14)
Horse	HPGDFGADAQGAMTK	751.8	1.5	−0.67	1268.5576 (y13) 1365.6103 (y14)
Pork	HPGDFGADAQGAMSK	744.8	1.5	1.34	1254.5419 (y13) 1351.5957 (y14)
Lamb	HPSDFGADAQGAMSK	759.8	1.5	0.79	1285.5525 (y13) 1381.6053 (y14)

Table 4. Other specific proteotypic peptides identified for selected mammalian meat species.

Protein: Myosin-1						
Species	**UniProt® Accession Number**	**Peptide Sequence**	**AA Position**	**Theoretical Mass (z = 2) — Observed Mass (z = 2)**	**Mass Accuracy (ppm)**	**R_t (min)**
Beef	Q9BE40	TLALLFSGPASG EAEGGPK	619–637	901.4702 — 901.4694	−0.89	16.8
Horse	Q8MJV0	TLALLFSGPASA DAEAGGK	619–638	888.4623 — 888.4620	−0.34	17.0
Pork	Q9TV61	TLAFLFTGAAGA DAEAGGGK	619–638	912.9600 — 912.9594	−0.66	17.4
Lamb	XM_00401 2706.1 (RefSeq)	TLAFLFSGAASA EAEGGGAK	619–638	927.9652 — 927.9650	−0.21	17.6

Protein: Myosin-2						
Species	**UniProt Accession Number**	**Peptide Sequence**	**AA Position**	**Theoretical Mass (z = 2) — Observed Mass (z = 2)**	**Mass Accuracy (ppm)**	**R_t (min)**
Beef	Q9BE41	TLAFLFSGTPTG DSEASGGTK	619–639	1022.4971 — 1022.4968	−0.29	16.4
Horse	Q8MJV1	TLALLFSGAQTA DAEAGGVK	617–636	960.5073 — 960.5070	−0.31	17.0
Pork	Q9TV63	TLAFLFSGAQ TGEAEAGGTK	619–638	978.4891 — 978.4894	−0.31	17.1
Lamb	XM_00401 2707.1 (RefSeq)	TLALLFSGTPTAE SEGSGTK	617–636	984.0020 — 984.0022	−0.20	16.5

Protein: ß-Haemoglobin						
Species	**UniProt Accession Number**	**Peptide Sequence**	**AA Position**	**Theoretical Mass (z = 2) — Observed Mass (z = 2)**	**Mass Accuracy (ppm)**	**R_t (min)**
Beef	P02070	FFESFGDLSTAD AVMNNPK	40–58	1045.4804 — 1045.4796	−0.77	16.9
Horse	P02062	FFDSFGDLSNPG AVMGNPK	42–60	1000.4646 — 1000.4637	−0.90	17.2
Pork	P02067	FFESFGDLSNAD AVMGNPK	2–60	1023.4673 — 1023.4670	−0.29	16.8
Lamb	P02075	FFEHFGDLSNAD AVMNNPK	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.

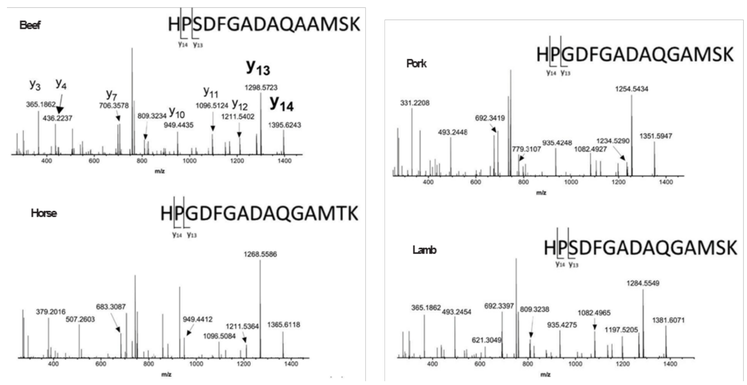


Figure 2. Extracted ion chromatograms for specific signature proteotypic peptide-fragment pairs. The data shows that each selected peptide-fragment pair was highly specific, which allowed it to perform accurate meat speciation. Additionally, very similar results were obtained using peptide precursor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

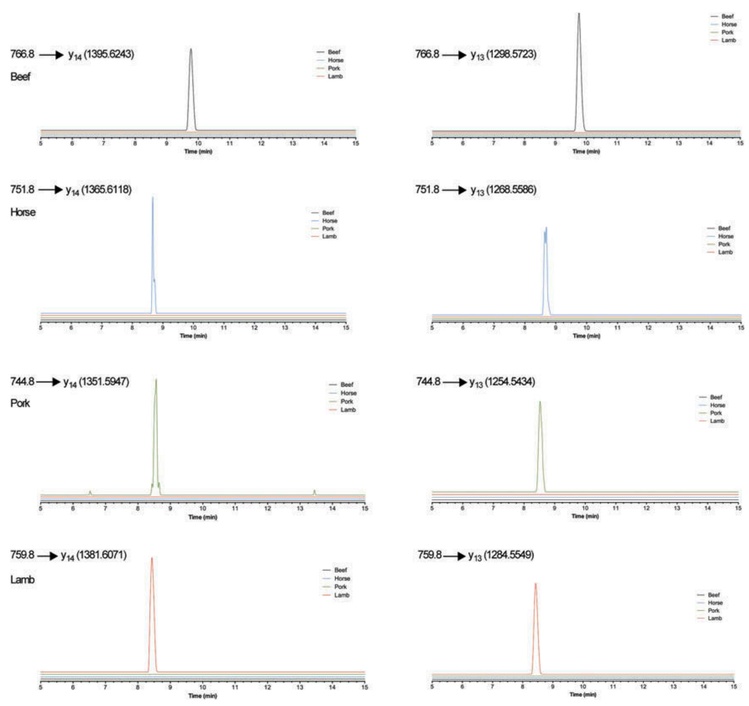


Figure 3A. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide fragment pairs. Chromatograms from meat samples spiked with 1% pork meat. (Extracted blank chromatograms are in blue.)

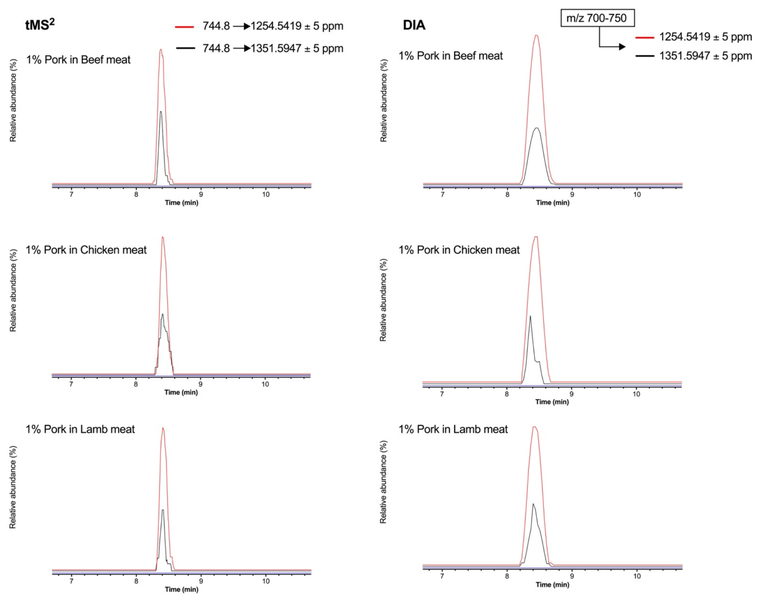
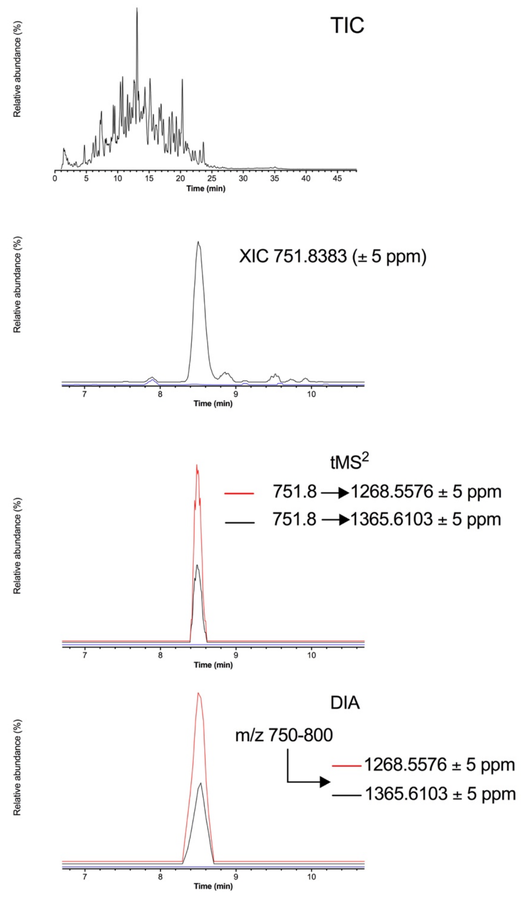


Figure 3B. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide-fragment pairs. Chromatograms from beef samples spiked with 1% horse meat. (Extracted blank chromatograms are in blue.)



CONCLUSIONS

- 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.

REFERENCE

1. Alberto Ruiz Orduna, Erik Husby, Charles T. Yang, Dipankar Ghosh & Francis Beaudry (2015) Assessment of meat authenticity using bioinformatics, targeted peptide biomarkers and high-resolution mass spectrometry, Food Additives & Contaminants: Part A, 32:10, 1709-1717, DOI: 10.1080/19440049.2015.1064173.

TRADEMARKS/LICENSING

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