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

Complete method: 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, DOI: 10.1080/19440049.2015.1064173

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	
MS:	Thermo Scientific Q Exactive benchtop quadrupole-Orbitrap mass spectrometer
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
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:	1.0×10^{-6}
Maximum IT:	100 ms
Isolation Window:	1.5 Da

HPLC Conditions	
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

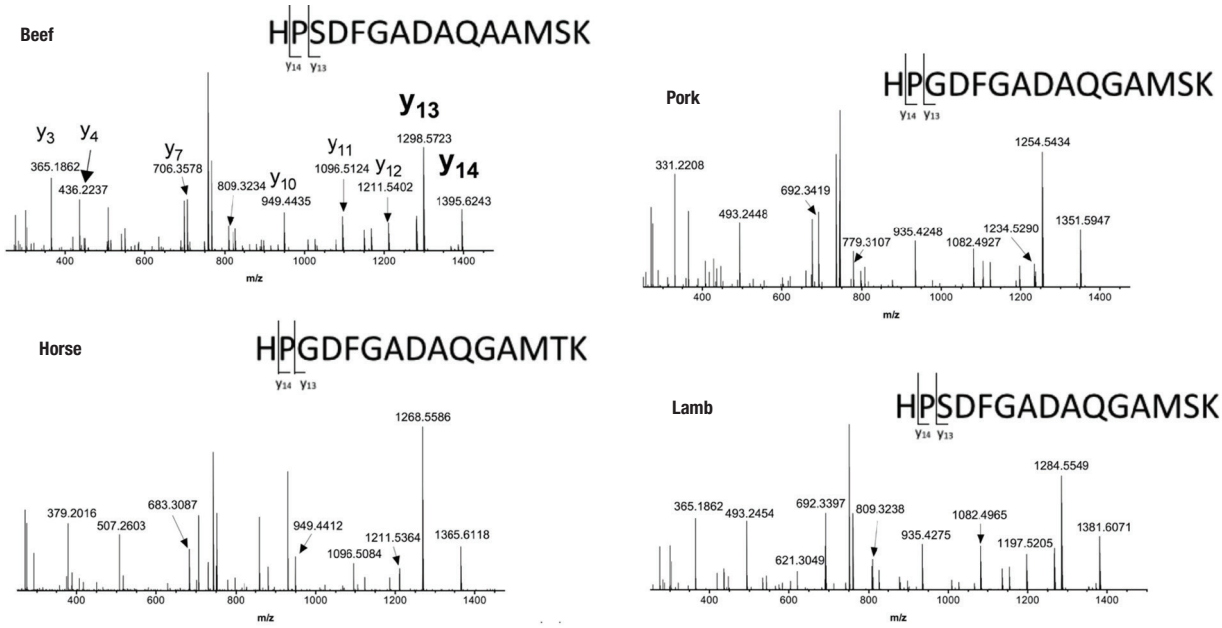

Figure 1. Product ion spectra of myoglobin proteotypic peptides (120–134). Fragment ion selectivity is preserved for y14 and y13 ions. These products can be used to produce specific product ion XICs for meat speciation.

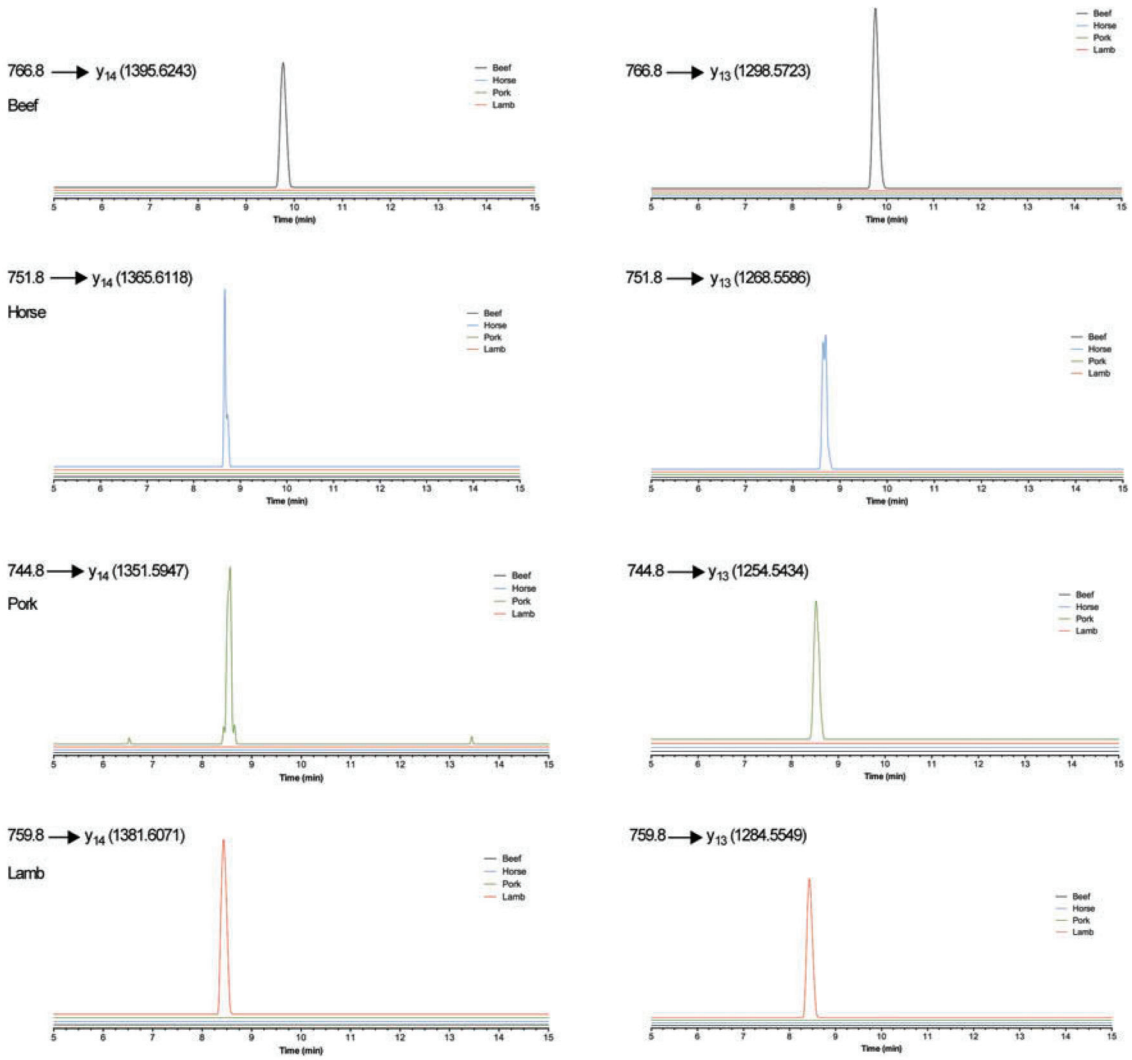

Figure 2. Extracted ion chromatograms for specific signature proteotypic peptide-fragment pairs. The data shows that each selected peptide-fragment pair was highly specific, which allowed it to perform accurate meat speciation. Additionally, very similar results were obtained using peptide precursor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

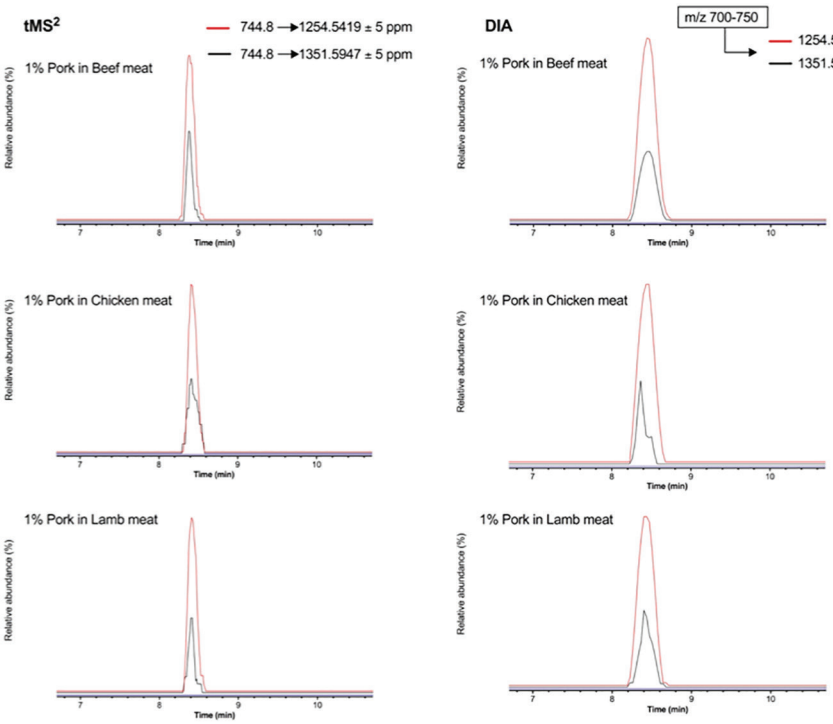

Figure 3A. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide-fragment pairs. Chromatograms from meat samples spiked with 1% pork meat. (Extracted blank chromatograms are in blue.)

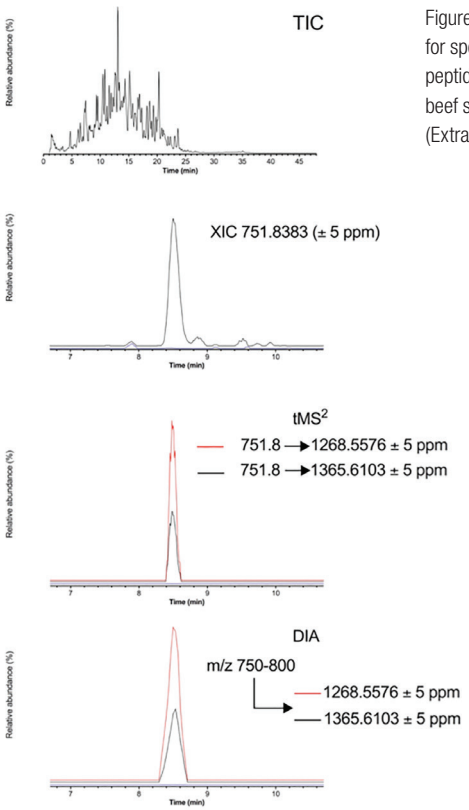

Figure 3B. Extracted-ion chromatograms (XICs) for specific signature myoglobin proteotypic peptide-fragment pairs. Chromatograms from beef samples spiked with 1% horse meat. (Extracted blank chromatograms are in blue.)

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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