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Cooked meat product analysis by LCMS/MS to determine between animal species using proteotypic peptide quantitative proteomic analysis

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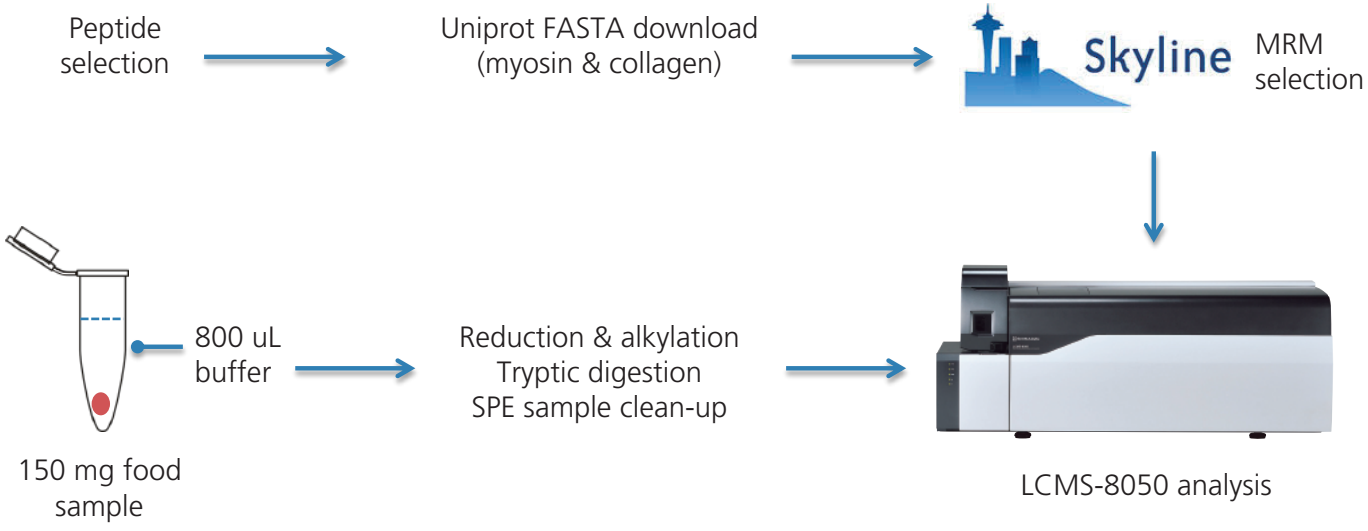
Introduction

With the emergence of new food safety challenges innovative analytical technologies are being developed to enable industry stakeholders and regulators to make sound scientific decisions. These advances have been made in response to changing regulations and needs and also to help maintain the safety of the food chain. In the case of unwanted or undeclared ingredients food authenticity testing is important component in many quality and safety programs. In 2013, horse meat issues received intense scrutiny and Polymerase Chain Reaction (PCR) techniques were used to screen for horse DNA in processed food samples. However, despite its widespread use in Europe for food fraud, PCR is problematic. DNA extraction kits are expensive and often require up to 8 h for the extraction, followed by cleanup and the PCR process. DNA is also prone to degradation especially in processed food. In contrast, mass spectrometry enables a promising approach to the detection of marker peptides in tryptic digests of horse, pig, beef and chicken with high sensitivity. In this work previously published proteotypic peptides were selected to detect horse, pork, beef and chicken using Skyline software (MacCoss Lab Software - University of Washington). These were used to interrogate Uniprot database FASTA download for myosin (43263 proteins) and collagen (44531 proteins) to determine no other species cross reactivity to pig myosin and collagen.

Method Development



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Materials and Methods

Sample preparation

Food samples were prepared by weighing 150mg into a microfuge tube, crushed using a micro-pestle then addition of 800uL extraction buffer (water containing 6M urea, 1M thiourea, 50mM Tris-HCl). Vigorous vortexing (2500rpm 15 sec), followed by centrifugation (13,000g, 10min), 100uL supernatant removed. Samples were reduced with addition of 30uL DTT (45mM) in 25mM ammonium bicarbonate 15min at 50C, then alkylated with addition of 30uL iodoacetamide (100mM) in 25mM ammonium bicarbonate 15min in darkness at room temperature, then diluted with 800uL 25mM ammonium bicarbonate. 30uL trypsin at 6.6uM in 25mM ammonium bicarbonate was added then incubated overnight at 37C. Sample clean-up following tryptic digestion was performed using off-line solid phase extraction (SPE) using Phenomenex Strata-X 33u polymeric reversed-phase (RP) cartridges filled with 30 mg/mL RP material. These were conditioned with 1mL methanol followed by 1mL acetonitrile, then equilibrated with 2mL water containing 5% acetonitrile + 0.1% formic acid. The sample was loaded, followed by cartridge washing (2mL water containing 5% acetonitrile + 0.1% formic acid) and elution (1mL 90% acetonitrile + 0.1% formic acid). Eluate was reduced by a centrifugal vacuum evaporation for 1h to approximately 50uL then increased to exactly 100uL with 3% acetonitrile + 0.1% formic acid.
Samples included: pork, chicken, beef and horse meat. Processed and mixed foodstuffs included: horse sausage, horse burger, beef haché, beef-chicken paste, beef-ham-chicken paste. Lamb mince was also analysed to represent a control sample. Cooked food was subjected to 200C for 1h.

Liquid chromatography		
UPLC	: Nexera LC system	
Analytical column	: Phenomenex Aeris C18 (150 x 2.1mm, 1.7um particle size).	
Column temp.	: 50 °C	
Injection cycle	: 2 µL injections	
Flow rate	: 0.3mL/minute	
Solvent A	: Water + 0.1% formic acid	
Solvent B	: Acetonitrile + 0.1% formic acid	
Binary Gradient	: Time (mins)	%B
	0	5
	3	5
	35	40
	37.5	100
	45	100
	45.1	5
	50	Stop

Mass spectrometry	
LC/MS/MS	: LCMS-8050
Ionisation mode	: Heated ESI
Polarity switching time	: 5 ms
Dwell times	: 47-117ms
Interface temperature	: 400 °C
Heating block	: 250 °C
Desolvation line	: 150 °C
Heating gas	: 15 L/min
Drying gas	: 5 L/min
Nebulising gas	: 2 L/min
CID gas pressure	: 350kPa

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Targeted peptides

Species	Protein	Accession	Peptide	RT (min)	Q1 prec.	MRM 1 (q)	MRM 2	MRM 3	MRM 4	CE (q)
Pig	MYH4	Q9TV62	TLAFLFAER	21.24	534.3 >	853.46	782.42	635.35	522.27	-18.9
	COL1A2	Q1T7B0	GVGAGPGPMGLMGPR	16.77	677.34 >	915.45	858.43	630.34	460.23	-34.6
Chick	MLE1	P02604	DQGTFEDFVEGLR	22.27	756.85 >	964.47	835.43	720.4	474.27	-27.8
	MLRS	P02609	GADPEDVIMGAFK	21.55	675.32 >	422.24	880.46	666.36	553.28	-28.5
			SFLEELLTTQCDR	24.20	777.88 >	723.31	949.48	836.39	622.26	-26.6
	MYSS	P13538	NLTEEMAVLDETIAK	22.90	838.92 >	789.44	959.54	888.5	676.35	-25.0
			VAEQELLDATER	13.87	687.35 >	591.27	817.44	704.36	476.25	-23.0
Bovin	MLRS	Q0P571	EASGPINFTVFLNMFGEK	30.60	667.66 >	985.48	838.41	725.33	480.25	-21.9
	CO1A1	P02453	GAPGADGPAGAPGTPGPQGIAGQR	30.79	686.34 >	883.47	729.4	601.34	-	-24.6
Horse	MYH1	Q8MJV0	TLALLFSGPASADAEAGGK	20.77	888.46 >	1030.48	973.46	805.37	532.27	-31.0
			VVETMQTMLDAEIR	19.90	818.41 >	488.28	948.48	847.43	603.31	-38.2
	MYH2	Q8MJV1	EFEIGNLQSK	14.72	582.8 >	646.35	888.48	759.44	475.29	-20.8

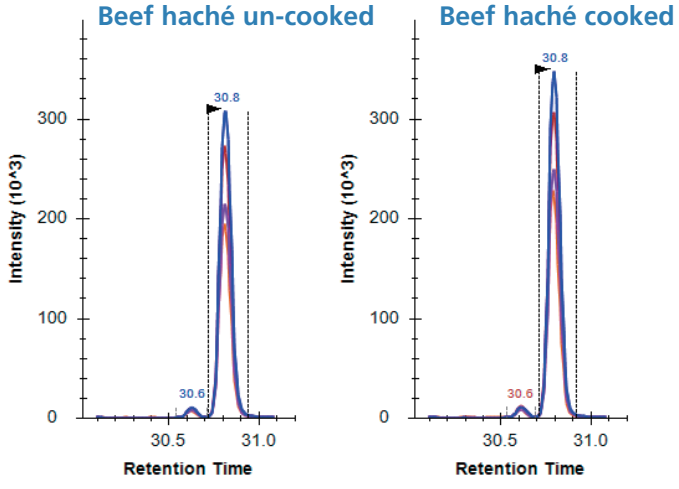
Acquisition parameters for food tryptic digest peptides: pig, chicken, bovine and horse. Q1 and Q3 bias used tuning file default settings, MS2 setting - Unit resolution, positive ionisation mode. Collison energy (CE) was set using predicted setting from Skyline software. CE optimisation was later performed once peptide RT was defined.

Results

Heat degradation effect

Heat degradation through cooking is a concern for food testing as this may impair measurement of target peptides and possibly reduce the apparent levels of animal material present. As a consequence of cooking however, the water and lipid content of the meat was significantly reduced ranging from 19% reduction in weight (chicken sausage) to 52% weight reduction (lamb mince). As a consequence of net weight loss in cooked food products, the anticipated signal loss through heat degradation was offset by net increase in concentration of target analyte.

Bovine myosin before and after cooking



Bovine myosin regulatory light chain 2, skeletal muscle isoform tryptic peptide: EASGPINFTVFLNMFGEK
Signal intensity from 150mg of beef haché increased from un-cooked to cooked however the process of cooking decreased the net weight by 29%.

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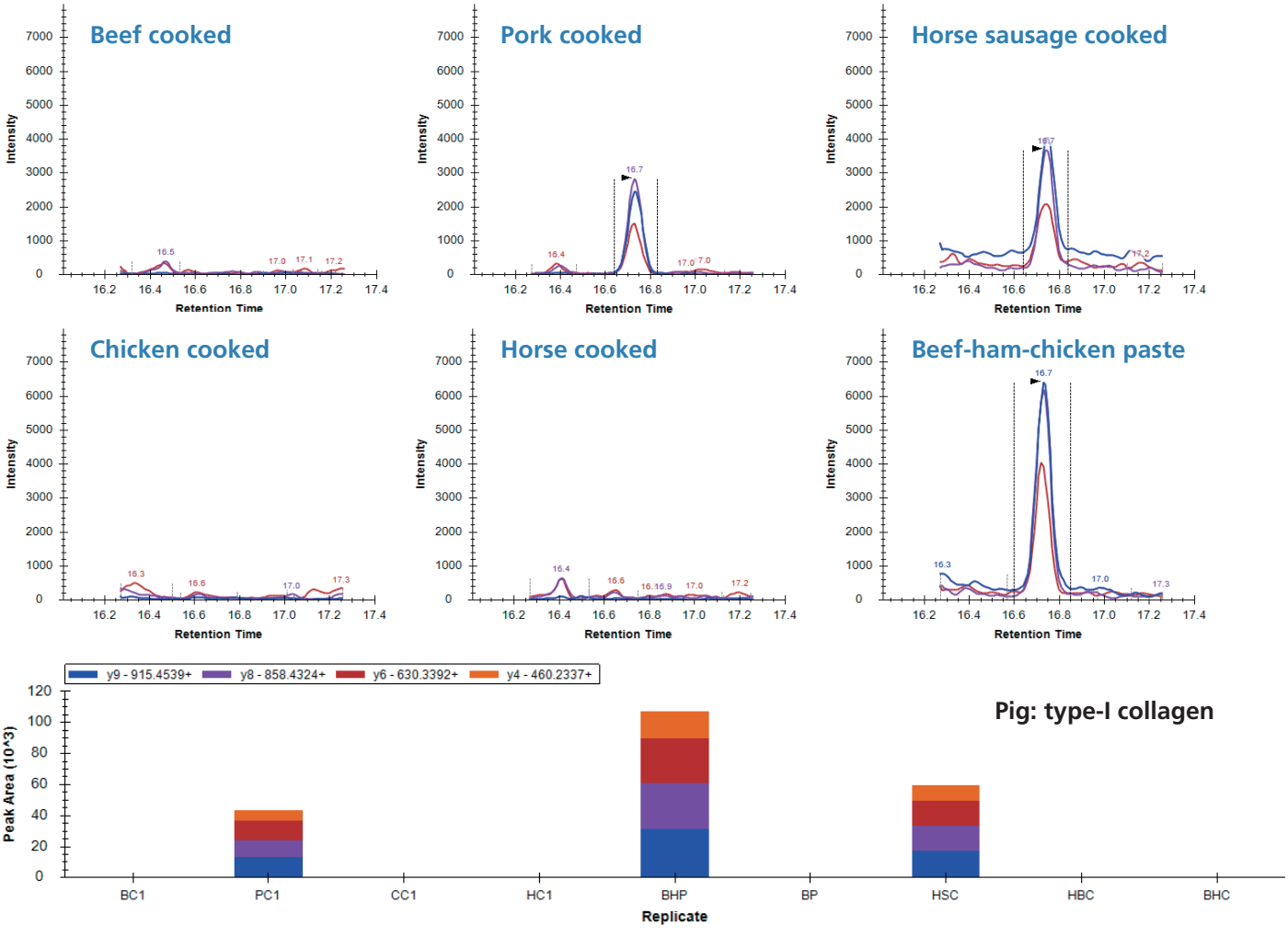
Sample drying / desiccation is not specified in food testing guidelines (LGC DNAS-13000036) however when testing precooked food products borderline quantities of contaminating material may be detected even if original raw ingredients were below a reporting threshold.

Likewise if heat degradation plays a significant part in reducing the limit of detection testing of uncooked food would be recommended in addition to testing of cooked food in order to prevent reportable levels of contamination being missed.

Pig: type I collagen

Measurement of pig collagen type I successfully detected pig collagen in mixed food types horse sausage and beef-ham-chicken sandwich paste.



Peak area comparison between different food types by type-I collagen alpha-2 chain tryptic peptide. (BC – beef cooked, PC – pork cooked, CC – chicken cooked, HC – horse cooked, BHP – beef-ham-chicken paste, BP – beef-chicken paste, HSC – horse sausage cooked, HBC – horse burger cooked, BHC – beef haché cooked).

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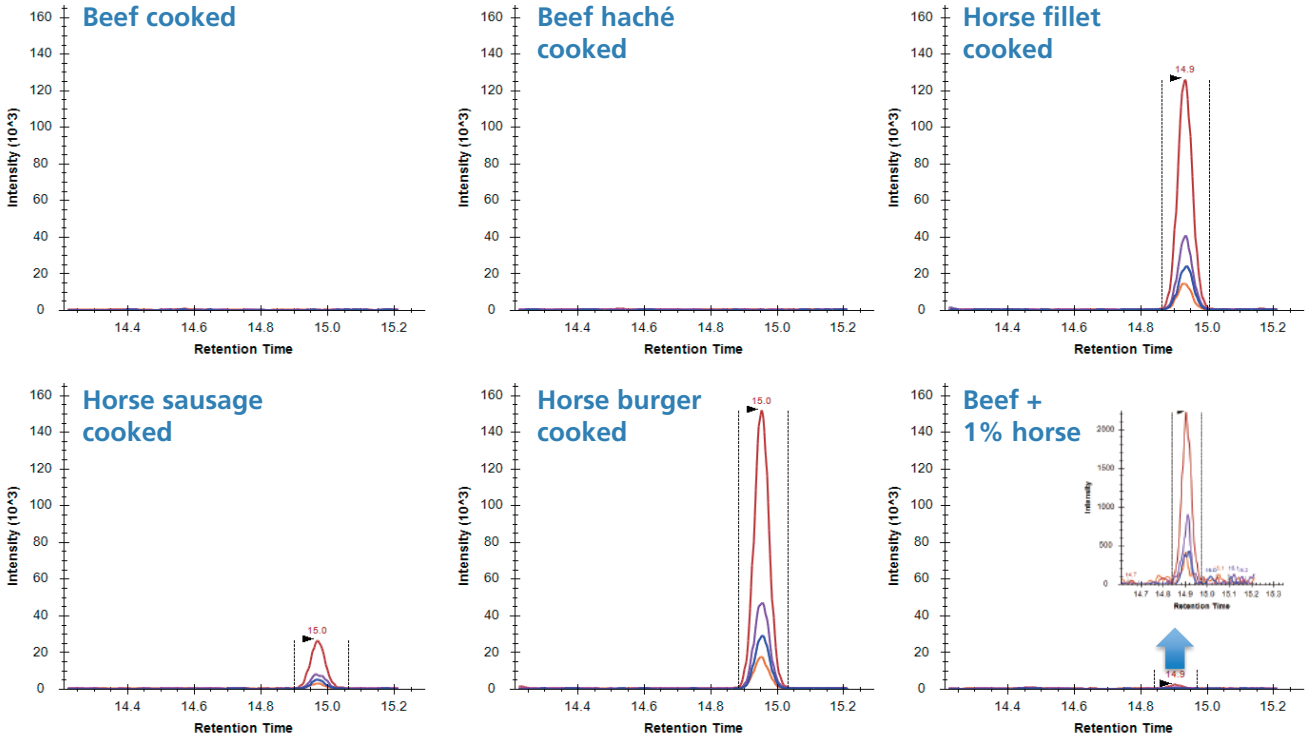
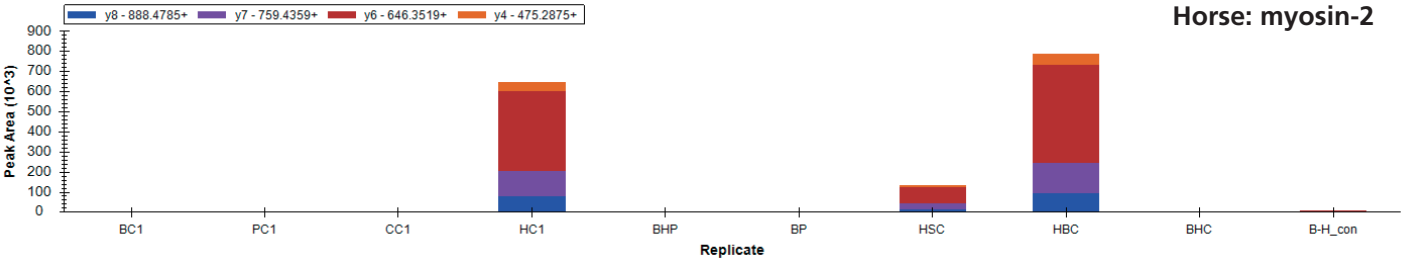
Horse myosin analysis



Proteotypic peptides from two different horse myosin proteins, myosin-1 and myosin-2, were measured from food samples. These successfully differentiated horse meat from other food types and was detected at 1% spiked level in beef sample.



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Conclusions

Current food authenticity testing focuses on animal speciation testing by PCR measurement of the mitochondrial gene cytochrome b. The strength of this approach offers any tissue type from an animal can be determined, however, it does not provide information on the type of animal tissue measured. Guidelines on food labelling and composition of meat products recommend limits on the percentage fat and connective tissue at 30% and 25% in pork products. In products containing meat from two or more species, measuring the level of connective tissue from more than one animal species adds further complexity to routine analysis. In these targeted proteomic experiments animal species can be determined in addition to measuring different proteins specific to a tissue type, therefore offering an added level of information not routinely performed in food testing.

References

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