DEVELOPMENT OF A LC-MS/MS METHOD FOR THE DETECTION OF SPECIES-SPECIFIC MUSCLE PEPTIDES IN PAPs

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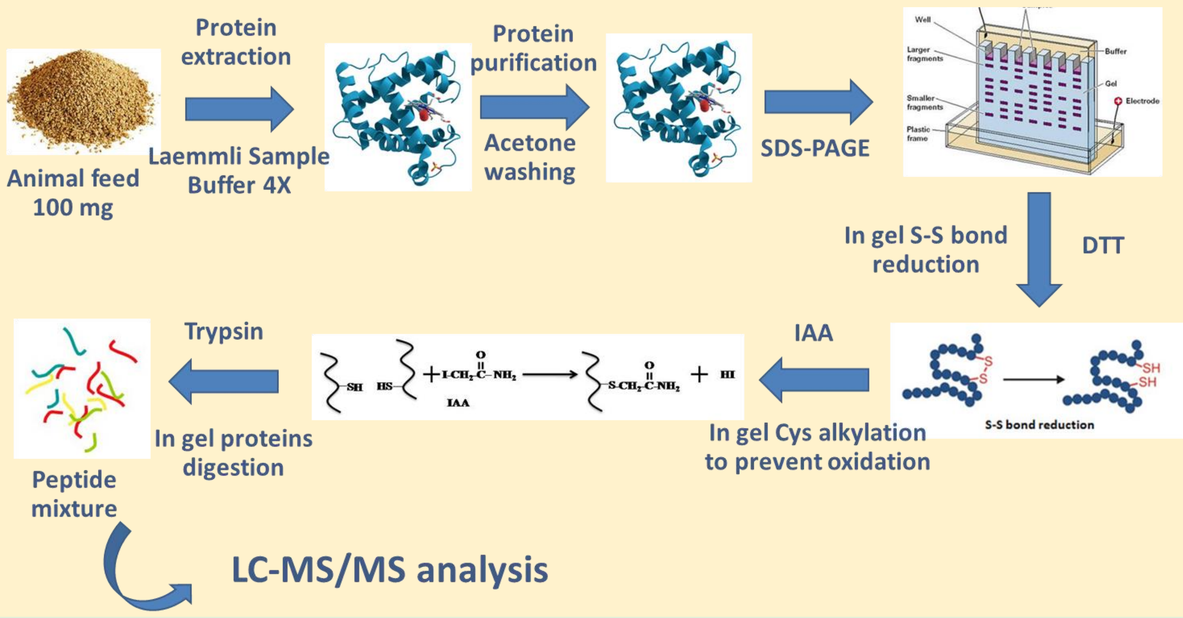
In the past, meat by-products have been associated to the exposure to the agent of the BSE, a prion disease [1]. In EU PAPs (Processed Animals Proteins) are subject to strict controls to avoid any possible exposure of ruminants to prions: in 2001 a total feed ban was applied to all farmed animals [2]. Regulation (EU) 51/2013 [3] lays down the official methods for determination of constituents of animal origin: banned PAPs can be detected by light microscopy (LM) and polymerase chain reaction (PCR). Nevertheless, even combined, sometimes these methods do not succeed in determining the taxonomic origin of the PAPs. In particular PCR analysis cannot distinguish between ruminant DNAs e.g. coming from muscle and bones from those originating from milk products, which are allowed to be used. Thus the addiction of milk products in feed could mask a possible presence of ruminant PAP, and leave the door open to potential frauds. Mass-spectrometry can be helpful in identifying peptides from specie-specific muscular proteins, since milk is an allowed ingredient in feed and PCR is not able to discriminate the origin of DNA.

HRMS IDENTIFICATION OF SPECIE-SPECIFIC PEPTIDES FROM BOVINE MUSCULAR PROTEINS

Species	Protein description	Peptide code	Peptide sequence	Peptide length	Molecular weight (g/mol)
Bovine	Desmin	TR-12	TSGGAGGLGALR	12	1016.11
	Vimentin	TR-14	TLYTSSPGGVYATR	14	1472.59
	Myoglobin	YK-16	YLEFISDAIIHVLHAK	16	1869.20

SAMPLE PREPARATION



LC-MS/MS method

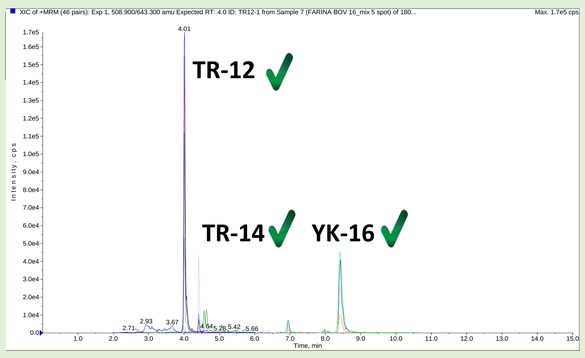
UPLC: Sciex ExionLC
Mass spectrometer: Sciex Qtrap 5500
Column: Phenomenex Kinetex C18 50x2.1 mm, 1.7 µm
Mobile phases: A = ACN + 0.1% HCOOH B = H_2O + 0.1% HCOOH
Flow = 350 µL/min
Vinj = 10 µL
Gradient:

Time (min)	% A	% B
0	3	97
0.5	3	97
10	45	55
10.2	95	5
12	95	5

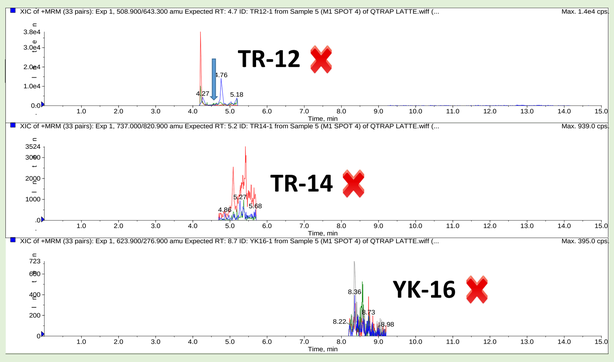
Peptide	Peptide code	RT (min)	MW (g/mol)	m/z (parent)	z	m/z (product)
Analytes	TR-12	4.06	1016.10	508.9	+2	643.3 – 828.4 – 416.2 - 771.4
	TR-14	4.46	1472.59	736.9	+2	215. 3 – 820.5 – 994.4 – 907.7
	YK-16	8.40	1869.18	624.0	+3	249.3 - 277.1 – 797.3
				468.2	+4	602.5
Labelled internal standards	TR-12 label	3.90	1030.10	516.0	+2	653.2 – 842.4 – 423.3 – 228.0
	TR-14 label	4.37	1479.59	740.6	+2	218.1 – 824.5 – 381.3 – 631.9
	YK-16 label	8.21	1883.18	628.6	+3	252.3 – 280.3
				471.8	+4	607.9 – 556.3
Process' internal standard (from Phr1 protein)	QR-15	4.60	1721.80	861.7	+2	740.8 - 852.5 - 242.2
				574.9	+3	214.1
	FK-14	4.61	1489.63	745.5	+2	938.1 – 277.1 - 552.0 - 249.0
	LK-12	5.95	1413.60	707.6	+2	783.2 – 233.2 – 1025.3
				472.1	+3	466.1

Bovine specific peptides were detected in PAP 100% bovine and were totally absent in milk products, as attended.
At high bovine PAP contamination (10% w/w) all the peptides are detected, while at low contamination level (0.1% w/w) only peptide YK-16 was identified.

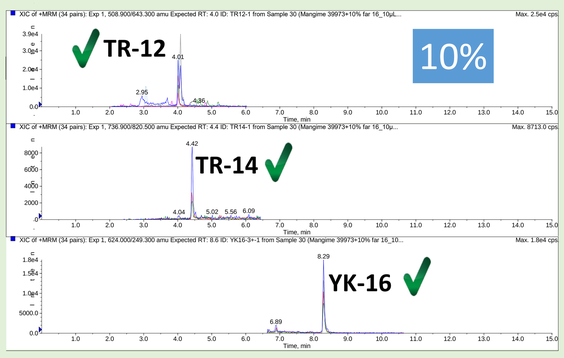
PAP 100% bovine

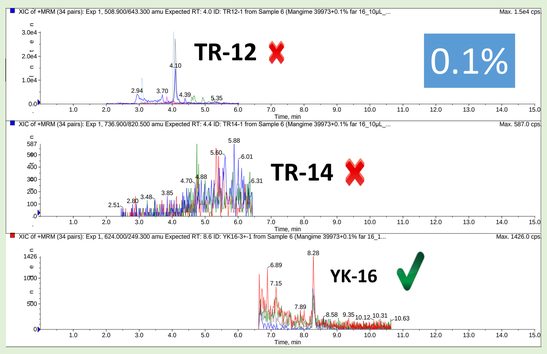


Milk product

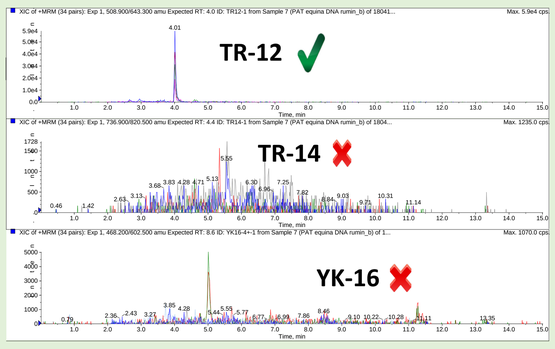


Animal feed spiked with bovine PAP 10% and 0.1% w/w respectively





HORSE PAP POSITIVE FOR AT PCR FOR RUMINANT DNA



In a real horse PAP sample coming from Argentina and positive for ruminant DNA, TR-12 peptide was detected. This is probably due to the rendering process utilized to obtain PAP.

CONCLUSION

The bovine peptides identified appear to be excellent biomarkers for detection of bovine PAP in animal feed. Their presence is an indisputable signature of bovine PAP. Moreover no possible cross reactivity appear to exist with dairy products. This could be of primary importance, as it will allow official and in house laboratories to detect unquestionably the presence of bovine PAP in feed.

References

[1] J.W. Wilesmith, G.A. Wells, M.P. Cranwell, J. B. Ryan, Veterinary Record, 123 (1988), 638–644
[2] European Commission, Official Journal of European Union, L147 (2001), 1-40
[3] European Commission, Official Journal of European Union, L20 (2013), 33–43